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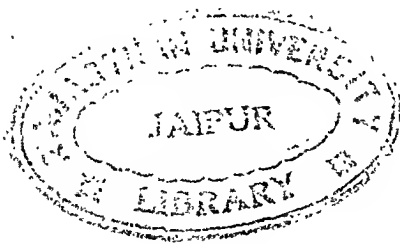
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## PATHOLOGICAL ANATOMY OF EXPERIMENTAL THROMBOPENIC PURPURA IN THE DOG \*

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The injection of a moderate dose of antiplatelet serum into a dog is followed by an acute attack of purpura lasting approximately 48 hours and ending in a spontaneous recovery. Larger doses are often fatal. The clinical manifestations of the experimental disease resemble closely those of thrombopenic purpura in man.<sup>27, 28</sup> This report is concerned with the gross and microscopic anatomical changes in the tissues and organs of dogs that died or were killed during or after one or more attacks of purpura induced in this manner.

From a survey of the literature one gains the impression that the lesions in thrombopenic purpura are neither specific nor constant.<sup>12, 22, 24</sup> Except for widespread hemorrhage, no significant pathological changes have been found common to all autopsied cases of thrombopenic purpura. Perhaps more extensive studies of the disease in man will show eventually a pathological picture, the evolutionary stages of which may resemble those found in our animals.

### METHODS AND MATERIALS

Twenty-five mongrel dogs from 8-20 kg. in weight and 1-3 years of age were employed. The antiplatelet serum was prepared

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in the rabbit by 6 intravenous injections of washed platelets isolated from 110-120 cc. of blood from normal dogs.<sup>27</sup> The anti-serum thus prepared is of approximately 1:32 to 1:64 intradermal purpurigenic titer. It was administered in varying doses intraperitoneally or intravenously.

*Experiment 1: (Fatal Group, 5 Animals):* Three were given a large dose of antiplatelet serum intraperitoneally (1-2 cc. per kg. body weight), and all died spontaneously 1, 1 and 3 days later, respectively. The other 2 animals died 1 and 2 days, respectively, after a moderate dose (0.1 cc. per kg. body weight).

*Experiment 2: (Serial Group, 12 Animals):* All were given a moderate dose of antiplatelet serum intraperitoneally (0.1 cc. per kg. body weight) and killed 1, 2, 3, 4, 5, 7, 10, 12, 13, 15, 17 and 34 days later respectively.

*Experiment 3: (Intravenous Dose Group, 4 Animals):* All were given a moderate dose of antiplatelet serum intravenously (0.1 cc. per kg. body weight) and killed 2, 17, 22 and 28 hours, respectively, afterwards.

*Experiment 4: (Multiple Dose Group, 2 Animals):* One received 2 large doses (1-2 cc. per kg. body weight) of antiplatelet serum intraperitoneally 5 days apart, and was killed 23 days after the last injection. The other received 7 moderate doses (0.1 cc. per kg. body weight) at 3 day intervals and was killed 33 days after the last injection.

*Experiment 5: (Control Group, 2 Animals):* These were given injections of normal rabbit serum in amounts equivalent to a moderate dose of antiplatelet serum. The injections were given intravenously and intraperitoneally, respectively, the animals being killed 5 and 24 hours later.

In the body of the text these experiments are designated as the fatal, serial, intravenous dose, multiple dose, and control groups. The expression "acute phase of purpura" indicates that period of the experimental disease lasting approximately 48 hours, during which the bleeding time is prolonged and hemorrhages develop at sites of trauma.

The dogs were killed over a period of 15 to 30 minutes by bleeding from the carotid artery after infiltrating the tissues locally with procaine. The autopsy was performed immediately afterwards and pieces of the various tissues were placed in fixing fluid as soon

as possible. The autopsy was complete in all respects with the exception of the spinal cord. The organs and structures of the abdominal, thoracic, cranial, orbital, oral and nasopharyngeal cavities were examined, together with the structures and organs of the face and neck, superficial lymph nodes, cutaneous, muscular, peripheral, nervous and osseous tissues, and bone marrow from ribs, sternum, femur, humerus and vertebra. When the animal died of the disease it was not always possible to carry out the autopsy immediately after death, but in the study of the tissue sections ample allowance was made for the possible complicating factor of postmortem autolysis. Likewise, due consideration was given the observation of others<sup>8</sup> that a few small hemorrhages may occur spontaneously during the agonal period when methods of killing somewhat analogous to ours are employed. That this is a small factor contributing to the production or aggravation of hemorrhagic manifestations is attested to in our control animals which showed only a few petechiae in the splenic mesentery, peritoneum of liver, renal capsule and mucosa of ileocecal junction. In the animals injected with the antiplatelet serum a few apparently fresh petechial hemorrhages, which occurred long past the acute phase of purpura, might thus have been agonal in character.

Pieces of tissue were fixed in a solution of formaldehyde (U. S. P., 1:10), and in Zenker's fluid; some were frozen and sectioned and stained for fat; the rest were embedded in paraffin and cut and stained by the following methods: hematoxylin and eosin, hematoxylin azure eosin, methylthionine chloride (U. S. P., methylene blue), van Gieson's and Mallory's connective tissue and Verhoeff's elastic tissue stains, and Mallory's potassium ferrocyanide stain.

Inasmuch as these methods of fixation and staining do not enable one to differentiate in tissue sections between platelets and platelet-like matter, no conclusions were drawn in regard to the changes and distribution of these elements throughout the body. Without resort to special methods,<sup>29</sup> platelets may be confused with precipitated plasma protein, pigment granules, disintegration products of blood cells, fibrils of connective tissue and pseudopodia of reticulum cells, fibrin strands and products of cytolysis.

## EXPERIMENTAL OBSERVATIONS

*Skin and Mucous Membranes:* Wine colored petechiae and hemorrhages of various sizes (2-40 mm. in diameter) occurred in the skin, especially about the mouth, ears, breast, groin, axilla and scalp, and at points of trauma, as cuts and needle punctures. Absorption of the hemorrhages, as indicated by fading, began shortly after their development and although a few were found as late as the 17th day, by the 4th day their number was materially reduced. There was a marked delay in this absorption in the fatal cases. In the early stages of purpura blood-stained discharges issued from the nose, mouth, rectum and vagina and the mucous membranes of these structures showed congestion, edema, petechiae and hemorrhages, the latter being numerous about the gum margins, base of tongue and tonsils. At this period there was usually a moderate degree of subcutaneous edema and in 1 animal that died from a large dose this was so marked that one foreleg was more than twice the size of its contralateral. Collections of edema fluid were distributed around hair follicles and small vessels of the papillary layer and caused separation of the connective tissue fibers of the skin. Hemorrhages were generally superficial in the derma but some extended deeply subcutaneously and along the ducts of the mammary gland. The erythrocytes were fairly uniformly distributed in parallel rows between the tissue fibers. Hemolysis and erythrophagocytosis occurred but fibrin was almost always absent. Occasionally, a slight inflammatory reaction was observed with small foci of necrosis in the overlying epidermis. In the tonsils of the animals of the intraperitoneal group the hemorrhages were small and confined to the stroma between the follicles, but in those of the intravenous doses group they were extensive both within and between the follicles.

*Skeletal Muscle:* Muscular hemorrhages developed in all animals within the first 17 days of the experimental period. The extravasated blood was distributed in longitudinal streaks parallel to the muscle fibers. During the acute phase of purpura the individual muscles were often notably increased in volume as a result of a combination of edema and hemorrhage. The areas most frequently involved were the tongue, musculature of the anterior thoracic and abdominal wall, diaphragm, and intercostal and axil-

lary muscles. Subperiosteal hemorrhages were frequently observed, especially over the calvarium and at the costochondral junction. In muscle the erythrocytes were either sparsely distributed or collected in large areas showing associated degeneration and necrosis of the muscle fibers and infiltration of inflammatory cells. In the tongue, extravasated blood appeared to spread dorsally, compressing the muscle fibers and blood vessels; the lymph vessels, however, were distended and filled with red staining material. Even in the absence of hemorrhage small groups of swollen hyalinized muscle fibers and minimal inflammatory changes were found in the extraocular, pharyngeal, laryngeal and tracheal muscles as late as 23 days after an injection.

*Heart and Aorta:* Cardiac lesions were observed in all animals. In the early cases the myocardium was soft, flabby, degenerated, edematous and mottled, dark red, gray and yellow. Fluid blood and different types of postmortem clots were found in the chambers of the heart, which were, in a few instances, distended to an unusual degree. During the first 12 days hemorrhages were common, particularly in the fatal cases. The pericardial fluid was sometimes blood-tinged and petechiae were numerous in the parietal pericardium and in the connective tissue around the aorta. In the epicardium petechiae and red linear streaks were frequently distributed along the course of the coronary vessels, the smaller branches of which were often congested. Subendocardial hemorrhages tended to occur at the base and free margins of the valve leaflets and superficially on the crests of the muscle bundles (Fig. 4), diminishing in the ventricles from base to apex. In 1 animal the entire auricular wall was hemorrhagic. In the course of time (7th to 34th days), these hemorrhages lost their bright color, turning brown or green, and underwent absorption. In the more recent lesions the erythrocytes were distributed singly and in groups loosely or tightly packed in and beneath the endocardium and epicardium, in the connective tissue septums, between individual muscle fibers, around blood vessels and in the lymphatic vessels. At various stages of absorption there were hemolysis, pigmentation, infiltration with leukocytes, erythrophagocytosis and occasionally a deposit of fibrin. Of particular interest were the endocardial bleb-like hemorrhages (Fig. 5) situated on the crests of the muscle bundles forming the chambers. The overlying endo-

thelial cells were tightly stretched but their continuity was nowhere broken. Beneath the blebs the connective tissue was edematous with focal hemorrhagic and inflammatory changes. All the animals studied showed some degree of swelling and vacuolization of the superficial muscle fibers near the endocardial surface. In several, during the acute phase of purpura, degenerative and necrotic changes involving groups of 3-20 muscle fibers were observed throughout the myocardium. In a fatal case there was a diffuse interstitial inflammatory reaction associated with extensive degenerative and necrotic changes in the muscle (Fig. 6). Another animal killed 23 days after the last of several doses showed extensive areas of chronic granulation tissue and fibrosis together with degenerative and necrotic changes in the muscle fibers. No changes were observed in the aorta.

*Lungs and Pleura:* In only a few of the animals the pleural cavity contained a slight excess of fluid and this was rarely blood-stained. During the acute stage the small intrathoracic vessels were congested and hemorrhages of varying extent were observed in the pulmonary tissue, coats of the great vessels, esophagus, trachea, bronchial lymph nodes and visceral and parietal pleura. In the fatal group and those killed early the pulmonary tissue was extensively hemorrhagic with varying grades of atelectasis, emphysema, edema and bronchopneumonia. In 1 animal there was a diffuse interstitial pneumonitis and in another a fatal massive submucous tracheal hemorrhage half obstructing the lumen.

Scattered over the visceral pleura of both lungs, especially along the margins, were numerous hemorrhagic areas, button-like or sometimes bleb-like, slightly elevated, and from 1-10 mm. in diameter (Fig. 10). Some presented a uniformly bright red stippled appearance with radiating streaks while in others the center was pale blue or purple with a surrounding red zone. These button-like lesions were most numerous during the first 5 days but were observed in various stages of regression and decolorization as late as 23 days after injection. The microscopic appearance of this lesion was that of any emphysematous, hemorrhagic pneumonic area embedded in atelectatic lung tissue. There were many eosinophils in the exudate. Involution was accompanied by erythrophagocytosis, hemolysis and pigmentation.

The most striking pulmonary lesion consisted of collar-like

extravasations of blood encircling bronchi and pulmonary vessels. It occurred regularly during the first 2 days. Not only was the lesion absent after the 2nd day, but there were few signs suggesting its previous presence. In some animals every branch and twig of the bronchial and blood vascular trees were involved but generally the lesion was confined to the medium sized subdivisions. The vessels and tubes thus surrounded showed slight compression but practically no irregularity or distortion of the lumens. When the thus affected bronchi were opened, the mucosa appeared dark bluish to black in the smaller ramifications. Around the blood vessels these hemorrhagic extravasations occupied a sharply delimited field in the adventitia and surrounding connective tissue, leaving the medial and intimal coats unaffected (Figs. 12 and 14). At the outer limits of the area was a thin fibrous and elastic membrane sharply delimiting the surrounding, slightly compressed pulmonary tissue. In the bronchi the ring was less often complete and erythrocytes were scattered throughout the remainder of the bronchial wall and in the lumen. The lesion almost never accompanied the smaller thin walled branches issuing from affected parent vessels; under these circumstances the collar-like extravasations stopped abruptly at the junction point. In the fresh state these lesions were composed of well preserved packed red cells, connective tissue fragments, and later macrophages showing active erythrophagocytosis. Widely spaced strands of connective tissue were arranged radially around the bronchus and circularly around the blood vessels. The bronchial mucous glands and occasionally the surface epithelium were compressed and necrotic.

On the 4th and 5th days the pulmonary vessels showed extensive clot formation and marked swelling of the endothelial cells. The pulmonary tissue, however, showed no evidence of recent infarction at this stage. Only 1 dog, at the 34th day, showed an organized infarct associated with canalized thrombosed blood vessels. Megakaryocytes were found in 3 animals of the serial group sacrificed on the 10th, 12th and 13th days and were exceedingly numerous in the small veins and capillaries. Their nuclei were hyperchromatic and distorted and processes of the cytoplasm radiated to the vessel wall, giving the cell a vacuolated appearance.

*Gastro-Intestinal Tract:* Hemorrhagic manifestations were abundant in the visceral and parietal peritoneum, omentum, mesen-

tery and alimentary tract in the acute phase of purpura and were observed, in diminishing frequency, up to 33 days. Blood tended to extravasate at the visceral attachments of the omentum and mesentery and along the veins (Fig. 2) which often exhibited ampulla-like dilatations. Intraperitoneal hemorrhagic effusions (from 10–250 cc.) were occasionally observed. The course traversed by the injection needle through the abdominal wall was surrounded by blood and the inner aspect of the abdominal wall at this point was marked by a localized area of fibrinohemorrhagic peritonitis which later organized with the formation of a large granulomatous mass. The majority of the hemorrhages in the esophagus, stomach and intestine were in the mucosa and submucosa but sometimes all coats were involved. Hemorrhages were most numerous in the mucous membrane of the pyloric ring, ileocecal valve, cecum and appendix (Fig. 1), and were absent from the Peyer's patches except in a single animal. Clusters of petechiae over an area 0.5–3 cm. were situated on the crest of the mucosal folds and sometimes in the grooves between the rugae (Fig. 3) or in a linear arrangement in the rectum. When there was hemorrhage in the submucosa or stroma of the intestinal glands the lining epithelial cells often showed degeneration, necrosis, desquamation and mitosis. Deeper hemorrhages were sometimes surrounded by necrotic muscle fibers and inflammatory cells.

Massive areas of hemorrhage involving large portions of the wall of the stomach or intestines were observed in 7 animals, 5 during the acute phase of purpura and 2 on the 5th and 13th days respectively (Fig. 2). The intestinal areas were situated 50–76 cm. from the pyloric orifice, measured 4–6 cm. in length, and were firm, mottled, dark red and swollen and occasionally obscured by local adhesions. All coats of the intestine were permeated with blood and the overlying peritoneum was elevated, stretched and torn in places. The mucous membrane was swollen, soft and deep red. Although the lumen of the intestine at the level of the lesion was usually markedly constricted, there was little or no change in its caliber above this point. Distally the lumen was distended and the contents blood-stained. In the stomach the lesion was massive and most of the hemorrhagic extravasation occurred into the submucosa and subserosa of the anterior wall. In 1 animal there was an associated intussusception involving the anterior wall of

the stomach, the ensheathing segment (intussusceptions) being formed by the hemorrhagic portion of the lesion into which the immediately proximal gastric wall was telescoped. Microscopically there was extensive necrosis of muscle fibers, nerve cells and overlying mucous membrane, marked inflammatory changes, and recent and canalized thrombi. The red blood cells were gradually broken down and absorbed. A later stage of this lesion was observed in a 13 day serial group dog; at this time most of the blood had been absorbed and large areas of muscle had been replaced by fibrous tissue.

*Thymus:* Petechiae, edema and venous congestion were observed in and around this organ chiefly during the first 7 days. Hemorrhage tended to occur in the interlobular and perithymic connective tissue, rarely within the lobule itself. Following these acute manifestations there was a marked proportionate numerical increase of reticular cells over thymocytes, and extensive pigmentation of phagocytes.

*Adrenals:* Only minor changes were noted in these organs. During the acute stage of purpura there was congestion of the vessels at the corticomedullary junction, hemorrhages in the surrounding areolar tissue and occasionally a few small hemorrhages in the medulla.

*Thyroid:* During the acute stage of purpura there were edema and congestion; small acinar collections of blood were observed in 2 dogs on the 5th and 12th days respectively.

*Salivary Glands:* Gross and microscopic study of these organs yielded negative results.

*Spleen:* Grossly not many changes were observed except for hemorrhages in the mesenteric attachment and, after the 10th day, follicular hyperplasia due chiefly to a marked increase in the large pale mononuclear cells of the germinal centers. In 2 animals killed on the 12th day and 13th day practically every follicle throughout the spleen contained a sharply circumscribed oval or crescentic red area composed of fairly well preserved erythrocytes and a few mononuclear phagocytes without associated changes in the surrounding pulp and sinusoids (Figs. 15 and 16). In the acute stage wine colored fluid blood issued from the cut surface. On the 5th day the large vessels contained cellular clots. From 4 to 15 days after an injection intravascular plugs were regularly



observed in the majority of the large and small vessels, frequently occluding their lumens to a considerable extent (Fig. 17). They were closely approximated to the vessel wall and were composed chiefly of large numbers of leukocytes with a variable but smaller proportion of erythrocytes and platelets, with fibrin in two instances and megakaryocytes in one. It was never possible with certainty to regard these "clots" as ante mortem, agonal or post-mortem. They were closely approximated to the vessel wall and platelets were arranged festoon-like as occurs in a thrombus. On the other hand, there was never any evidence of coincident infarction or congestion of the splenic tissue, no changes in the vessel wall along the line of approximation and no organization or canalization of the masses.

Megakaryocytes appeared from the 3rd to the 15th day in increasing numbers, averaging sometimes from 12 to 30 per low power field. They were of the young and old type and were located most frequently near the trabeculae, in groups of 2 or 3, in association with erythropoietic foci, or in the lumens of vessels. In 1 dog, dying 1 day after a single large dose, there were several megakaryocytes encircled by large mononuclear cells, as in the bone marrow.

*Liver:* The hepatic changes differed considerably, depending upon the route of administration of the serum. In the intravenous group the liver was large, edematous and mottled, deep red and gray. Microscopically about the portal radicles there were hemorrhage, intense inflammatory changes, extreme distention of lymphatic vessels (Fig. 8) and compression and necrosis of bile ducts and adjacent parenchyma. The inflammatory cells consisted chiefly of polymorphonuclear leukocytes and eosinophils. The central and sublobular veins were constricted and throughout the lobule there was extensive hyaline and fibrinous thrombosis of the sinusoids with sporadic necrosis of the parenchyma and hemorrhage. Clusters of erythrocytes and large spherical hyaline droplets were observed in rounded spaces interpreted as bile canaliculi between hepatic cells. There were petechiae and hemorrhages in the serosa of the bile ducts and gall bladder, and the wall of the latter was thickened by an exudative inflammatory reaction (Fig. 13).

In the animals that received the serum intraperitoneally the

acute changes resembled those just described but were milder in character and of gradual development and regression. Other changes included blood in the bile ducts, portal vein thrombi and, from the 7th to the 12th day, small foci of erythropoiesis. As a rule central veins were collapsed and inconspicuous. Small hyaline thrombi were occasionally observed in the sinusoids and a few small hemorrhages about portal radicles. Parenchymal necrosis occurred throughout the first 15 days, being extensive in the first 3 days and located chiefly in the inner two-thirds of the lobule, frequently in association with sinusoidal thrombi and hemorrhages. Regressive changes were accompanied by hepatic cell regeneration which was especially active on the 4th day. The lesions usually underwent complete resolution; in only 1 animal, killed at the 34th day, was there a moderate portal fibrosis.

*Pancreas:* No lesions were observed except slight congestion and a few interstitial petechiae.

*Genito-Urinary Tract:* During the first 4 days the kidneys showed a variable degree of nephrosis and a few petechiae in the capsule and perirenal fat. Subserous, submucous and intramural petechiae and hemorrhages were frequently found in the bladder, especially in the region of the trigone. In the male dogs hemorrhages occurred in the tunica vaginalis, epididymis, vas deferens and testicle during the phase of purpura. The testicular hemorrhages were large and wedge-shaped, extending from the center of the lobule to the periphery. In the female sex organs only a few petechiae were noted in the peritoneum of the ovary and in the uterine mucosa. Vaginal bleeding has been referred to above.

*Brain:* Intracranial hemorrhage of some degree, usually minimal, was observed during all stages of the experiments, being perhaps in some instances agonal in origin. Hemorrhage was most marked in the fatal cases, yet even in these no extensive extravasations occurred into the brain substance or into the ventricular cavities. Petechiae and larger purpuric patches occurred over the inner surface of the skull, in and beneath the dura, pia and arachnoid, especially along the sulci and Pacchionian bodies, subependymally, in the chorioid plexus and occasionally also in the parenchyma. Superficial parenchymal hemorrhages resulted from extension of the subpial extravasations, whereas the deeper ones were peri-

vascular in location and numbered as many as 3 per low power field. Immediately around the latter there was a narrow zone of tissue degeneration. In the animals of the intravenous injection group the cerebral tissue was edematous, congested, and of soft consistence.

*Pituitary:* No visible changes were observed aside from vascular congestion during the acute stage of the purpura.

*Lymph Nodes:* In the acute stage the nodes were large, soft and succulent, and contained red areas outside, in and beneath the capsule, and on the cut surface. Externally these red areas corresponded either to hemorrhages or to distended afferent lymph vessels packed with erythrocytes (Figs. 7 and 9). These red areas were noted in varying intensity in different nodes up until the 17th day. However, many nodes began to show a brownish cast after the 4th day. Within the node were tightly packed erythrocytes in distended lymph sinuses, chiefly in the medulla. The impression was gained that the red cells were brought by afferent lymph vessels, passed quickly through the cortical sinuses, and stagnated in the medulla. The process was always orderly even when large numbers of red cells were present. With marked distention of the sinusoids the intervening medullary cords became atrophic. The erythrocytes were cleared away by phagocytosis and the deposition of pigment, followed by an increase in lymphocytes in the still distended sinuses. Pigmentation was greatest between the 10th and 17th days. By the 34th day the histiocytes were comparatively inactive, shrunken and had long thin processes. The compression atrophy of the lymphoid tissue during the acute phase was followed (from the 10th to the 34th day) by hyperplasia of the medullary cord and follicles, the latter being composed of large pale cells with a reticular nucleus. In all the sections examined only 3 megakaryocytes were found (on the 12th and 34th days).

*Bone Marrow:* Changes in the megakaryocytes were the principal feature observed in this tissue, thus reflecting the close association between platelets and these giant cells. Hyperplasia and varying predominance in quantity of erythroid and myeloid elements did not differ significantly from the controls. Microscopic hemorrhages developed only in the fatal cases and, in association with thrombi, in a 2 hour intravenous group dog. The

megakaryocytes showed combined regressive and regenerative features both in the series as a whole and in the same animal. Pyknosis, karyorrhexis and karyolysis, marked irregularity in cell outline and increased granularity of the cytoplasm involving 50 per cent or more of these giant cells predominated from 2 hours to 15 days; later than this necrosis was rare. What appeared to be invasion by polymorphonuclear leukocytes was present at all stages, being most marked at the 13th day when 30-40 per cent of the megakaryocytes were so affected; as many as 9 polymorphonuclears were counted in a single megakaryocyte. Young megakaryocytes were observed in greatest number 2 hours after an intravenous injection. In the serial group they increased numerically from the 2nd to the 13th day (Fig. 20), after which they began to decline. A few forms interpreted as mitotic figures were observed in all stages of the experiments.

Another mechanism for the formation of bone marrow giant cells distinct from the generally proposed one was suggested by a curious grouping of mononuclear cells about single necrotic megakaryocytes in a circular fashion resembling a rosette figure. These figures were observed in 5 dogs at the 2nd and 3rd day in the serial group and at 17, 22 and 28 hours in the intravenous group. The marrow of these animals showed a diminution in the myeloid and erythroid elements and a diffuse increase in a mononuclear type of cell with a large, pale vesicular nucleus and relatively little cytoplasm. Many of these mononuclear cells were also arranged focally around megakaryocytes in a rosette form outside the cell border, completely isolating them from other cells (Figs. 18 and 21). The nuclei of the encircled megakaryocytes were pyknotic, irregularly shaped and indented, and the cytoplasm vacuolated. Many phases of this process were observed from mere accumulation of a few cells around apparently viable megakaryocytes to the point where the nucleus of the latter existed only in outline encircled by several cell rows. In some marrow specimens the majority of the megakaryocytes were necrotic and encircled by these mononuclear cells. In addition to these formations there were numerous giant cells with concentrically arranged nuclei (Fig. 19), with hypertrophied and apparently fused nuclei, representing perhaps transitional stages in the formation of mature megakaryocytes.

## COMMENT

The lesions of experimental purpura differ somewhat according to the method employed for inducing it (intravenous or intraperitoneal injection) and with the stage of the disease. When the antiplatelet serum is administered intravenously the hepatic changes indicate an anaphylactic response resembling that produced by other types of cytotoxic antisera.<sup>25, 32</sup> Following intraperitoneal injection, however, the serum is absorbed more slowly, the number of platelets in the circulation diminishes more gradually, and the resulting tissue changes are probably more comparable with those in naturally occurring thrombopenias in man. The clinical and pathological manifestations of the experimental disease show a uniform evolution, the stages of which may be conveniently divided as follows: (1) acute stage (1-5th day), exhibiting thrombopenia, prolonged bleeding time and, in the tissues, hemorrhage, edema and pigment deposition; (2) intermediate stage (5-10th day), showing a rising platelet count, short bleeding time, and multiple vascular thrombi in various organs, principally in the spleen; (3) reactive stage (after the 10th day), characterized by a high blood platelet count, follicular hemorrhages in the spleen and hyperplastic changes in the bone marrow, spleen, lymph nodes, thymus and Peyer's patches of the ileum.

There have been few studies of the lesions of thrombopenic purpura in man in which proper consideration was given to such features as the date of onset and duration of the disease, the number of attacks, the degree of thrombopenia, the distribution and intensity of the hemorrhages, and the mechanism of the period of recovery, and so on. There are sporadic indications that the evolutionary changes of experimental purpura in the dog are duplicated, to a certain extent, in man. Thus in a patient with thrombopenia complicating lupus erythematosus, Baehr and co-workers<sup>3</sup> noted occlusion of nearly all precapillary arterioles with platelet thrombi. Klemperer<sup>23</sup> observed thrombi in the arteries of all organs of a girl with thrombopenic purpura who died after a splenectomy and, in another patient, reticulum cell hyperplasia of the spleen with widening of Billroth's cords. Arrigoni and Calabresi<sup>1</sup> observed cellular adherent thrombi in the veins and hyperplasia of the reticuloendothelial cells in the spleens of their

patients. Perivascular extravasations of blood from the vessels of the brain forming ring-like hemorrhages are characteristic of "cerebral purpura" complicating arsphenamine therapy.<sup>15</sup> The occurrence of follicular hemorrhages in the spleen has been observed by us and others.<sup>5,7</sup> The frequency of hepatic edema in thrombopenic purpura has been recently emphasized; in 55 per cent of such cases studied by Keschner and Klemperer<sup>22</sup> there was collection of fluid between the liver cell cords and the walls of sinusoids. Acute abdominal symptoms may complicate purpura in man,<sup>20</sup> sometimes eventuating in a laparotomy<sup>4</sup>; in these cases there are often petechiae and ecchymoses in the mesentery and the appendix is enlarged, edematous and hemorrhagic, a counterpart of the manifestations of early experimental purpura in the dog. Intestinal intussusception with obstruction is not a rare complication in man.<sup>16</sup>

From this evidence it seems likely that the lesions of thrombopenic purpura, as a group at least, are more specific than is generally believed. It is admittedly difficult, however, to establish these correlations solely from human pathological material. Most patients with primary thrombopenic purpura recover spontaneously from the acute attack and in fatal cases little or no allowance is generally made for the duration of the disease in evaluating tissue changes. Moreover, if, as frequently happens, the thrombopenia is secondary, its lesions are masked to a variable degree by the primary condition, be it leukemia, aplastic anemia, carcinoma, or tuberculosis or other infections. Recurrent attacks at short intervals result in superimposed lesions and a more complex histopathological picture. By making serial observations in experimental animals over the period of evolution of a single attack, some of these pitfalls may be avoided.

Certain deductions may be made from a study of the distribution and extent of the hemorrhagic manifestations and their relationship to preexisting and concurrent lesions, trauma, mobility and vascularity of parts. Trauma plays a major rôle in conditioning the skin for the immediate or delayed development of local hemorrhages.<sup>13,27</sup> In animals injected with antiplatelet serum hemorrhages develop at points in the skin traumatized within 4 days preceding the injection, as well as during the acute stage of purpura.<sup>27</sup> Essentially similar conditions obtain in the internal

viscera. For example, no hemorrhages were found in and about long-standing chronic granulomatous lesions of a parasitic nature in parenchymatous organs, on serous surfaces or in the musculature, especially of the tongue; at points of attachment of intestinal worms hemorrhages were sometimes present, sometimes absent, depending in all probability on whether the trauma was recent or remote. A tendency exists for hemorrhages to occur in organs that normally during life are undergoing movement almost constantly or being subjected to external or internal changes in pressure, even while the subject is at rest. Thus, hemorrhages were common in the lung, thymus, heart, intestine, mesentery, omentum and urinary bladder; they were minimal in relatively immobile or protected organs such as the bone marrow, pancreas, adrenal, liver, kidney and brain. Local trauma, stress and spontaneous lesions, both recent and remote, seem, therefore, to play an important part in the localization of hemorrhages in purpura. Examples of normal internal stresses that may constitute mild forms of trauma are the alternating intrapleural and intracardiac pressure, action of the heart, intestinal peristalsis, and contraction and distention of the urinary bladder. Under normal conditions these stresses are adequately compensated and no ill effects result; in thrombopenic purpura or in scurvy<sup>2,33</sup> compensation fails and hemorrhage may result. If, as is frequently maintained, the effects of the antiplatelet serum were due principally to its injurious action on blood vessels, then one would expect to find the hemorrhages distributed more in proportion to the vascularity of the organs and to occur frequently, for example, in the adrenals, kidney or epididymis. Such was not the case, however, for in these organs hemorrhages were rarely seen.

The mechanism of hemorrhage in purpura, the rôle played by the vessels, and the point in the vascular tree through which blood escapes, have long been the subject of speculation. According to Unna,<sup>30</sup> the cutaneous extravasations of blood originate principally from ruptured venules in the subpapillary layer. We have been unable to confirm this observation. The relationship between an area of extravasated blood and any given vessel may be purely coincidental. Curious appearances may be presented in single histological sections by such structures as the neurovascular glomus body. Breaks in the walls of small and large blood vessels

were repeatedly observed in the material from both the purpuric and the control animals and were in many instances obvious artefacts. Any hypothesis designed to explain the escape of blood into the tissues must take into account the fact that under physiological conditions erythrocytes may traverse the apparently uninjured capillary wall.<sup>11</sup> It follows that an exaggeration of this mechanism in purpura may lead to increased diapedesis of erythrocytes, independent of injury to the endothelium or rupture of the wall of the vessel.

The most unusual type of hemorrhagic lesion encountered in this series of animals occurred in the lung during the first days of thrombopenia in the form of peribronchial or perivascular circular accumulations of blood. We interpret these lesions as representing blood within the adventitial and perivascular lymphatic vessels. In other words, the erythrocytes thus situated occupy a position analogous to that of exogenous pigment granules and associated phagocytic cells in certain forms of pneumoconiosis (Fig. 11). The blood was absent from these areas after 48 hours and there was no evidence of fading such as ordinarily occurs in most hemorrhages. The distended, filled lymphatic vessels may be seen clearly when this lesion is observed in newborn puppies with purpura induced by antiplatelet serum. In the adult dog the outlines of the lymphatic vessels are less readily demonstrable. Several factors may be contributory to the production of this lesion: increased absorption by the lymphatics of red cells from hemorrhagic areas in the periphery of the lung, exaggerated diapedesis of erythrocytes, or lymph stasis due to overburdened regional lymph nodes with retrograde flow of lymph. That the lymphatics play a dominant rôle in the pathogenesis of this lesion is attested by the fact that afferent lymphatic vessels to lymph nodes were likewise often greatly distended with blood (Figs. 7 and 9). Against the possibility that the blood in the collar-like lesion issued from the vessel so surrounded or from the adjacent pulmonary tissue is the sharply demarcated character of the lesion (Fig. 12). Furthermore, the usual failure of hemorrhages in general to show absorption in the center, in the animals that died from an acute attack, may be another sign of the retardation or failure of lymphatic circulation. Congestion and stasis of lymphatic vessels may also account for the edema which is a



constant finding in the acute phase of purpura. Marked distention of lymph vessels was observed not only about lymph nodes in the perivascular pulmonary tissue but also in the portal radicles of the liver and in loose connective tissue, especially of the mesentery and omentum.

A connection may exist between these collar-like pulmonary hemorrhages in the dog with induced purpura, and the occurrence of clinical purpura in cases of lymphangitic carcinomatosis of the lungs. The latter condition has been recently reviewed by Jarcho.<sup>19</sup> Almost all of the thoroughly studied cases of carcinoma with purpura have been characterized by lymphangitic carcinomatosis of the lung.<sup>19</sup> Despite the fact that many such cases also exhibit neoplastic permeation of the bone marrow, the accompanying thrombopenia cannot be linked to destruction or disappearance of megakaryocytes; on the other hand, practically all such cases of purpura show neoplastic permeation of the lymph vessels of the lung. In view of the evidence for the pulmonary origin of platelets,<sup>18</sup> one may venture to speculate regarding the effect of lymphangitic carcinomatosis of the lung on thrombopoiesis. The primary tumor in these cases is usually situated in the stomach.<sup>19</sup> It has been suggested that tumor cells emanating from this organ proceed to the abdominal lymph nodes, thence to the mediastinal nodes, and thus engender a stasis of the lymph in the lungs. Similar routes may have been traversed by products of the local purpuric lesion in the region of the stomachs of our animals injected intraperitoneally with antiplatelet serum, thus perhaps explaining the similarity in the morphology of these two pulmonary lesions.

None of the spleens in our animals presented enlargement, congestion or internal hemorrhages during the acute phase of purpura despite the fact that this organ displays a certain degree of movement. Killing by exsanguination may, of course, have reduced the size of a previously engorged spleen. A palpable spleen is occasionally found clinically in thrombopenic purpura and has been attributed to hyperplasia or to accumulation of blood in the pulp by either sinusoidal congestion or massive extravasation. In our animals follicular hemorrhages occurred on the 12th and 13th day, when the number of platelets in the peripheral blood reaches its highest level. We have observed at autopsy an

identical lesion in a female subject in whom there were widespread hemorrhages associated with a long-standing clinical erythro-leukothrombopenia. Carnot, Lafitte and Fiehrer<sup>7</sup> reported a similar case of profuse bleeding checked by removal of the spleen in which follicular hemorrhages were found, the intervening pulp being only moderately congested but otherwise normal. This was the sole lesion found in 1 of 11 spleens from patients with thrombopenic purpura examined by Brown and Elliott.<sup>5</sup> In a group of dogs injected with massive doses of parathyroid hormone follicular hemorrhages of the spleen were a constant finding, along with hemorrhagic manifestations in other parts of the body.<sup>6</sup> The etiology and mechanism of development of these splenic hemorrhages is obscure. It is difficult to understand their occurrence in the dog at a time when the animal has recovered from an acute attack of purpura, the mean bleeding time being shorter than normal and the number of platelets in the blood increased. Since the spleen plays an important rôle in the distribution, utilization and, indirectly, in the formation of platelets, the occurrence of these follicular hemorrhages might be linked, in the dog, to the excessive number of platelets, but in man such association seems unlikely. The attack of bleeding in the patient studied by Carnot and coworkers had lasted 6 weeks before splenectomy, in the one reported by Brown and Elliot, 3 weeks, and our patient died after several months hospitalization during which the platelets at repeated determinations did not at any given time go beyond 50,000 per cmm.

Qualitative changes in the megakaryocytes have been stressed as being equally as important in influencing the production of platelets as quantitative deficiencies. Thus, megakaryocytes of the young or lymphoid type are considered to possess limited capacity for thrombopoiesis.<sup>20, 14, 17</sup> It is frequently impossible to establish in human thrombopenic purpura any significant direct correlation between the number of megakaryocytes of the bone marrow and the circulating blood platelets. Such a correlation was suggested by our experimental results in the adult dog, the tissue megakaryocytes undergoing marked degenerative changes during the acute stage and seeming to become more numerous after the animal recovered from the attack. At this period young megakaryocytes and some forms suggesting the mode of formation

of these bone marrow giant cells were common. That megakaryocytes may be formed by fusion of adjacent cells is maintained by Di Guglielmo<sup>9</sup> and Fabris.<sup>10</sup> In animals rendered thrombopenic with pyrodine the latter found megakaryocytes resembling closely those we describe as "rosette" forms. Great caution, however, is necessary in drawing conclusions regarding the morphological appearance of megakaryocytes in single histological sections. From a study of serial sections and reconstruction models of individual megakaryocytes Kaufman<sup>21</sup> pointed out that their small end segments may be interpreted as mononuclear cells or young megakaryocytes. When their pseudopodia encircle other cells, like leukocytes, the impression of phagocytosis or invasion may be gained from simple transverse sections.

In our experience the most striking demonstration of the histogenetic relation between the megakaryocytes and the platelet was in 2 day old pups that received a small dose of antiplatelet serum.\* Normally in the liver, spleen and bone marrow of such pups there are large numbers of megakaryocytes as well as foci of erythropoiesis and leukopoiesis. The intraperitoneal injection of antiplatelet serum is followed within 24 hours by the practically complete disappearance of megakaryocytes from these three tissues, leaving the foci of erythropoiesis and leukopoiesis relatively unchanged. Megakaryocytes reappear in the bone marrow 3 days following the injection, are fairly numerous by the 5th day and increase in number subsequently.

### SUMMARY

The clinical and pathological manifestations of experimental purpura as produced with antiplatelet serum in the dog undergo a uniform evolution, the stages of which may be conveniently divided as follows: (1) acute stage (1st-5th days), exhibiting thrombopenia, prolonged bleeding time and, in the tissues, hemorrhage, edema and pigment deposition; (2) intermediate stage (5th-10th days), during which there is a rising platelet count, short bleeding time, and multiple vascular thrombi in various organs, principally in the spleen; (3) reactive stage (after the 10th day), characterized by a high blood platelet count and

\* Many other lesions of experimental purpura in pups differ materially from those in adult dogs and will form the subject of a separate report.

hyperplastic changes in the bone marrow, spleen, lymph nodes, thymus and Peyer's patches of the ileum. There are indications that these evolutionary changes of the experimental disease are duplicated to a certain extent in spontaneously occurring thrombopenic purpura of man.

The intensity and distribution of the hemorrhages seem to be largely conditioned by the trauma and degree of internal or external stress normally undergone by the part.

Signs of congestion and partial obstruction in the circulation of the lymph are evident during the acute phase of purpura. Lymph vessels in walls of the blood vessels and bronchi of the lung, in the heart muscle, afferent lymphatics and sinuses of lymph nodes draining hemorrhagic areas become greatly distended with blood. Edema and failure of resorption of hemorrhages may be the result of failure of the lymphatic circulation.

Follicular hemorrhage is one of the outstanding lesions in the spleen; it appears only in animals recovering from the attack of purpura and immediately after the time when the number of platelets usually reaches its highest level. Marked degenerative changes take place in the megakaryocytes of the bone marrow during the acute stage; groups of mononuclear cells surround degenerated megakaryocytes, the group eventually enlarging and resembling the mature forms of the giant cells. Intraperitoneal injection of antiplatelet serum in 2 day old pups was followed by the almost complete disappearance of the usually numerous megakaryocytes from the liver, spleen and bone marrow.

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## DESCRIPTION OF PLATES

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### PLATE I

FIG. 1. The vermiform appendix of a dog killed 3 days after a moderate dose of antiplatelet serum intraperitoneally.

FIG. 2. Large intestine of a dog that died 1 day after the injection of a large dose of antiplatelet serum intraperitoneally with rupture and partial obstruction of the intestine.

FIG. 3. Lower part of the esophagus, stomach and small intestine of a dog killed 3 days after a moderate dose of antiplatelet serum injected intraperitoneally.



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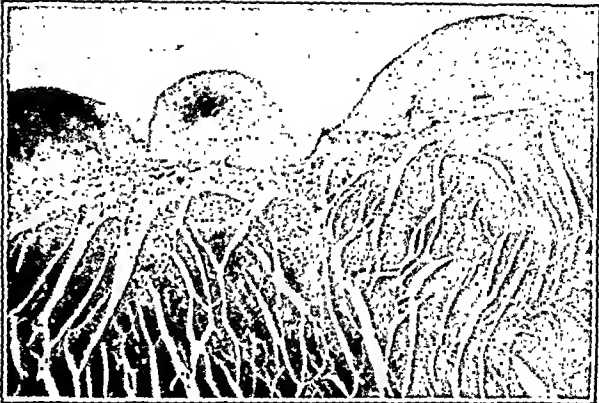


## PLATE 2

- FIG. 4. A portion of the heart of a dog that died 2 days after the injection of a moderate dose of the serum intraperitoneally.
- FIG. 5. Section from the same heart illustrated in Figure 4 showing subendocardial "blood blebs."
- FIG. 6. Section from the heart of a dog that died 2 days after the intraperitoneal injection of a moderate dose of antiplatelet serum showing cellular infiltration about necrotic myocardial fibers.
- FIG. 7. Section of lymph node of a dog that died 2 days after injection of a moderate dose of antiplatelet serum intraperitoneally. An afferent lymph vessel adjacent to the cortical sinus is greatly distended with red and white cells.
- FIG. 8. Section of the liver of a dog killed 22 hours after the injection of a moderate dose of the serum intravenously. Marked dilatation of the periportal lymphatics is seen.
- FIG. 9. Section of lymph node of a dog that died 2 days after injection of a moderate dose of antiplatelet serum intraperitoneally. Marked engorgement of an afferent lymph vessel is present. Almost all of the medulla of this lymph node was replaced by blood.



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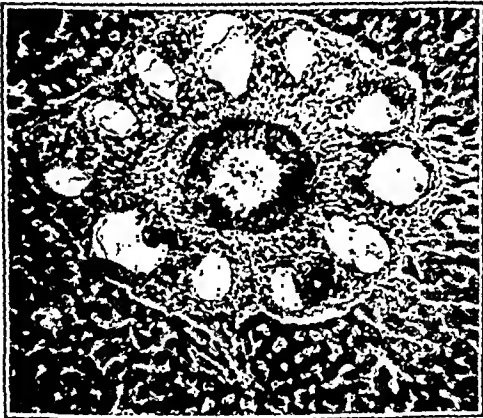
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Tocantins and Stewart

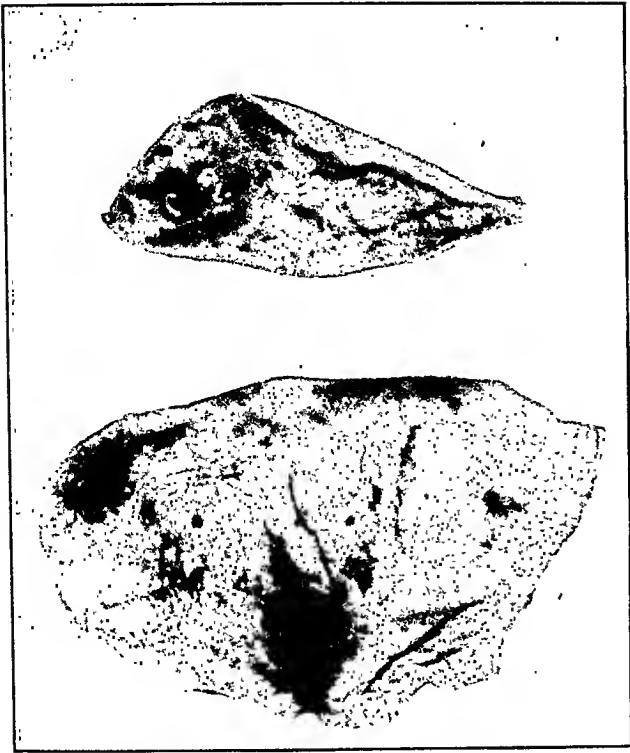


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Experimental Thrombopenic Purpura

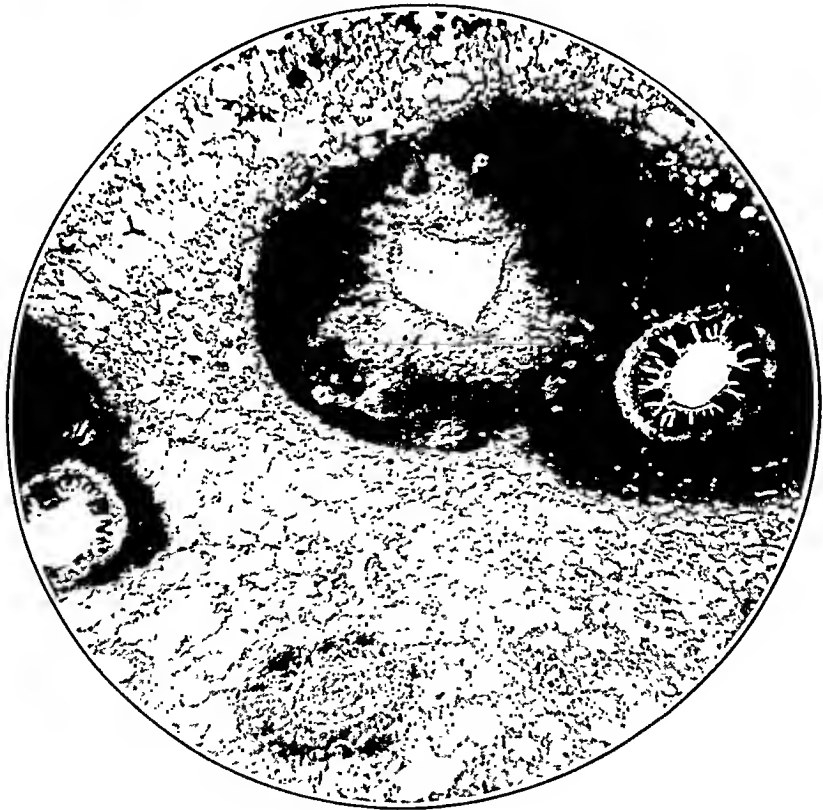
PLATE 3

- FIG. 10. The cut and external surfaces respectively of the lung of a dog that died 2 days after injection of a moderate dose of antiplatelet serum intraperitoneally.
- FIG. 11. Low power view of a section of the lung of a dog killed 2 days after a moderate dose of antiplatelet serum intraperitoneally. Massive collar-like accumulations of blood about vessels and bronchi are present.
- FIG. 12. Section from the same lung illustrated in Figure 11. High power view of the collar-like collections of blood. The intervening tissue is almost wholly free of blood.



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Tocantins and Stewart

Experimental Thrombopenic Purpura



#### PLATE 4

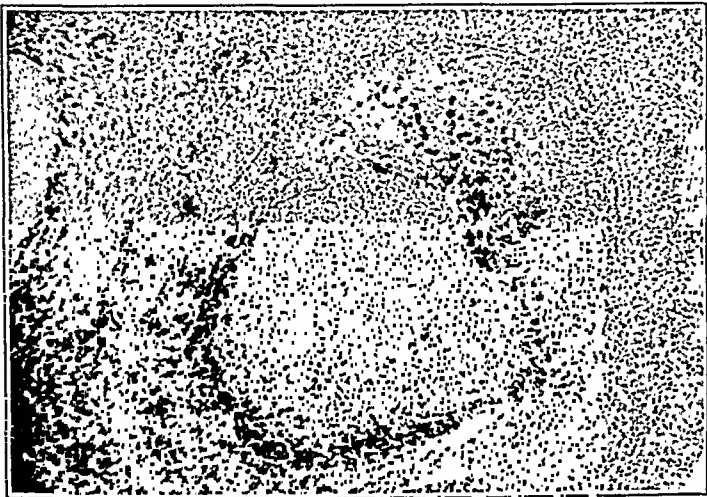
- FIG. 13. Section from the gall bladder of a dog killed 2 hours after the injection of a moderate dose of the serum intravenously. Edema of the wall, especially of the serosa, is seen.
- FIG. 14. Section from lung of a dog that died 1 day after injection of a large dose of antiplatelet serum intraperitoneally. A massive, collar-like perivascular collection of blood is present which appears to be in the adventitia of the vessel.
- FIG. 15. Section from the spleen of a dog killed 13 days after the injection of a moderate dose intraperitoneally showing a perifollicular elliptical hemorrhage around a large germinal center.
- FIG. 16. Section from the spleen of a dog killed 12 days after an injection of a moderate dose of antiplatelet serum intraperitoneally showing an intrafollicular hemorrhage.
- FIG. 17. Section from the spleen of a dog killed 7 days after the injection of a moderate dose intraperitoneally. An intravascular plug made up of red and white cells and agglutinated platelets is seen.



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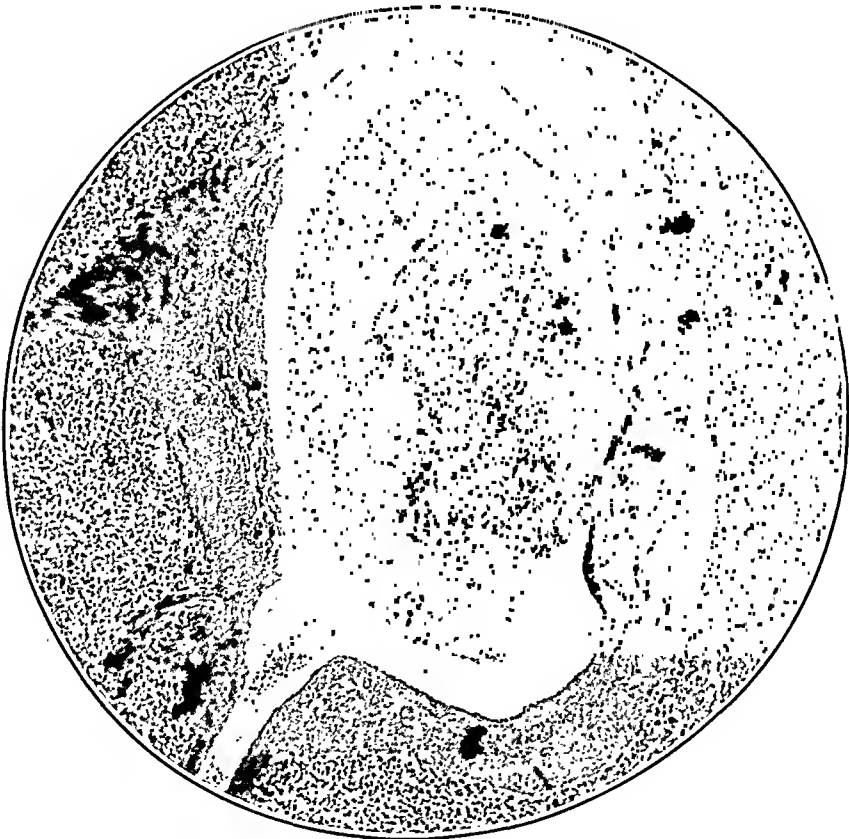
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PLATE 5

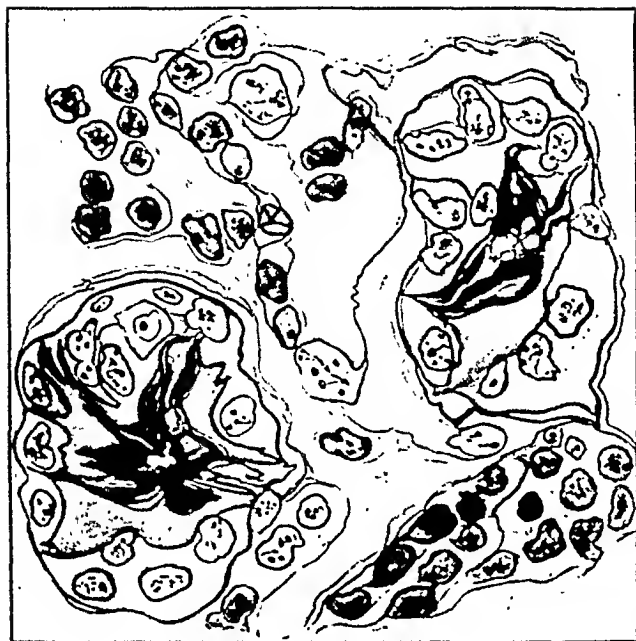
FIG. 18. Camera lucida drawing of a section of the bone marrow of the femur of a dog killed 3 days after the injection of a moderate dose intraperitoneally. Megakaryocytes surrounded by mononuclear cells in rosette fashion are seen.

FIG. 19. Camera lucida drawing of a section of the bone marrow of a dog killed 12 days after the injection of a moderate dose of serum intraperitoneally showing megakaryocytes with a circular arrangement of nuclear pieces.

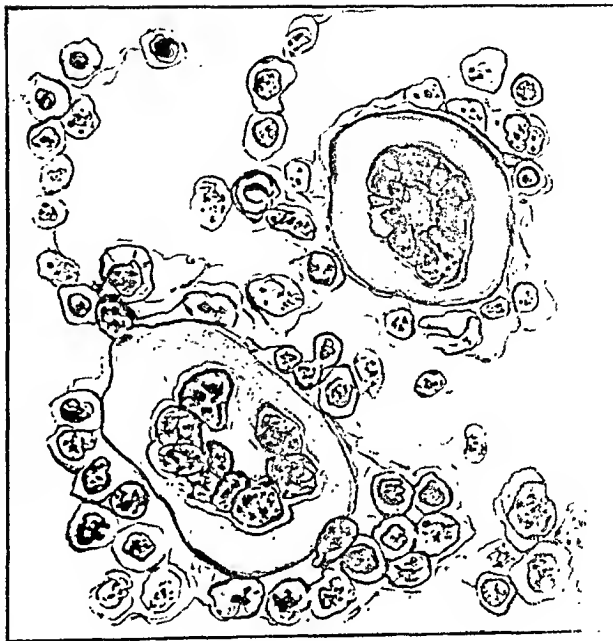
FIG. 20. Section of the bone marrow of the femur of a dog killed 10 days after the injection of a moderate dose intraperitoneally. Numerous megakaryocytes of various types are present.

FIG. 21. Section of bone marrow of a dog killed 4 days after the injection of a moderate dose intraperitoneally showing a megakaryocyte surrounded by mononuclear cells.

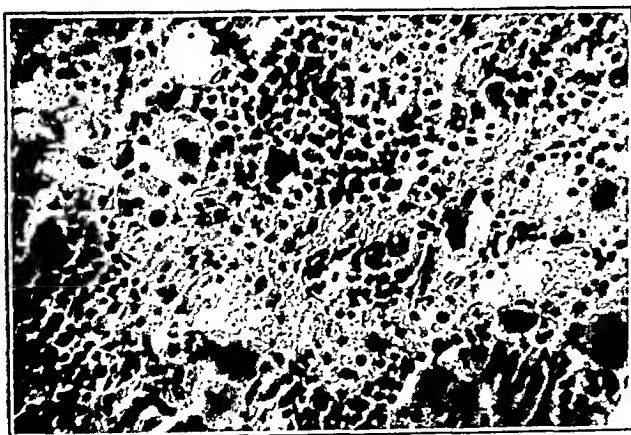




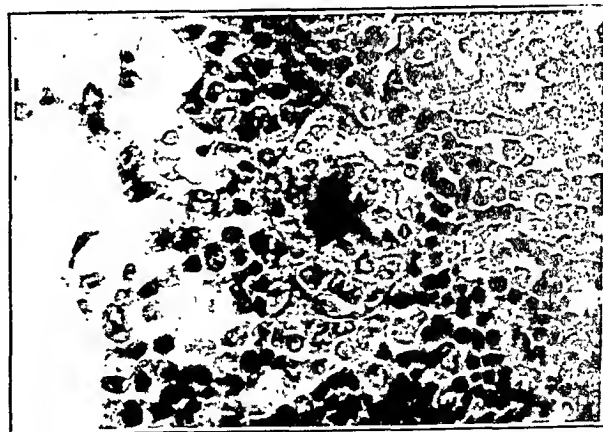
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Tocantins and Stewart

Experimental Thrombopenic Purpura





# PATHOLOGICAL CHANGES IN THE HEART, SKELETAL MUSCULATURE AND LIVER IN RABBITS TREATED WITH INSULIN IN SHOCK DOSAGE \*

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The main interest in pathological changes produced by the insulin shock treatment since it was introduced by Sakel<sup>1</sup> as a cure for schizophrenia has been centered on the brain. Comparative studies on experimental insulin and anoxic shock revealed a surprisingly close similarity in the symptomatology produced by these two procedures.<sup>2</sup> Rabbits treated with a series of anoxic shocks showed quite definite changes in various parenchymatous organs, in addition to changes in the central nervous system. Therefore, a study of the same organs was undertaken with rabbits exposed to insulin shock. This report concerns mainly the changes found in the heart, skeletal musculature and the liver.

## METHOD

Twenty nine rabbits were treated in 4 series with various doses of insulin. Lilly's Iletin was used. Whenever "units" of insulin are referred to, "units per kilo body weight" is meant. The insulin was administered in the morning to rabbits which were not fasting but were, however, not fed on that day until the termination of the experiment. The insulin effect was interrupted between 6 and 12 hours after its administration by feeding cabbage or carrots and pellets. No glucose was given for the termination of the shock except to the rabbits of the first 2 series, which received a subcutaneous injection of 10 cc. of 40 per cent glucose on the 1st day of the experiment just prior to their feeding. In a few other instances glucose was given on various days of the experiment only when the rabbits were obviously unable to recover spontaneously.

The doses of insulin were chosen so that all rabbits of the first 3 series were severely affected, while the doses given to the 4th series remained just below the threshold that had produced deep

\* Received for publication September 19, 1938.

coma and convulsions in any instance in the preceding series. Charts 1 to 3 show the doses of insulin administered to single animals of Series I to III, and indicate by broken lines the days on which convulsions were observed. They show also whether the animal died spontaneously or was sacrificed by air embolism or by suffocation in a chamber filled with pure nitrogen.

The 4th series consisted of 8 rabbits treated 28 times with insulin doses of from 0.74 to 1.3 units during a period of 41 days. In addition to this insulin treatment, during the same period these rabbits were exposed 4 times to anoxic shocks, *i.e.* comparatively short periods of gradually increasing deficiency of oxygen in a mixture with nitrogen until the respiration became gasping or ceased entirely. Any accumulation of carbon dioxide in the gas chamber was prevented by continuous flow of the gas mixture. The insulin treatment produced only a very slight apathy in the animals in this series. The anoxic shocks were administered on the 1st or 2nd day, the 8th or 9th, the 17th and the 41st day after the beginning of the insulin treatment. The first 2 anoxic shocks were begun 5 hours after the injection of insulin, the last 2 about 2 hours after the injection. Their duration was from 30 to 60 minutes. Each rabbit was seized by violent convulsions of about 1 minute's duration following the termination of 1 or 2 of the anoxic shocks. At the end of the 1st anoxic shock on the 1st day insulin was injected 1 rabbit was sacrificed, 3 days following the 2nd anoxic shock a 2nd rabbit, and at the end of the 3rd anoxic shock a 3rd rabbit was sacrificed. After the termination of the treatment on the 41st day the remaining 5 rabbits were kept alive for a period of 50 days for full recovery. During this period they showed no pathological symptoms whatsoever. They gained weight from 50 to 300 gm., and were finally sacrificed by suffocation in a nitrogen chamber.

The blood sugar was controlled by the Folin-Wu method on samples of blood withdrawn from the ear vein. In addition, blood was also withdrawn from the hepatic vein immediately after death or several times successively up to 20 minutes after death.

A careful gross examination of each animal was performed after death. Material to be used for frozen sections (fat stains) or the usual staining methods after embedding in paraffin was fixed in formalin (U.S.P.). Thin slices of tissue were fixed in absolute

alcohol for Best's glycogen method which was employed on celloidin or paraffin-celloidin sections.

## RESULTS

Individual rabbits displayed a great variability in their response to insulin, as is best reflected by the charts showing by what doses

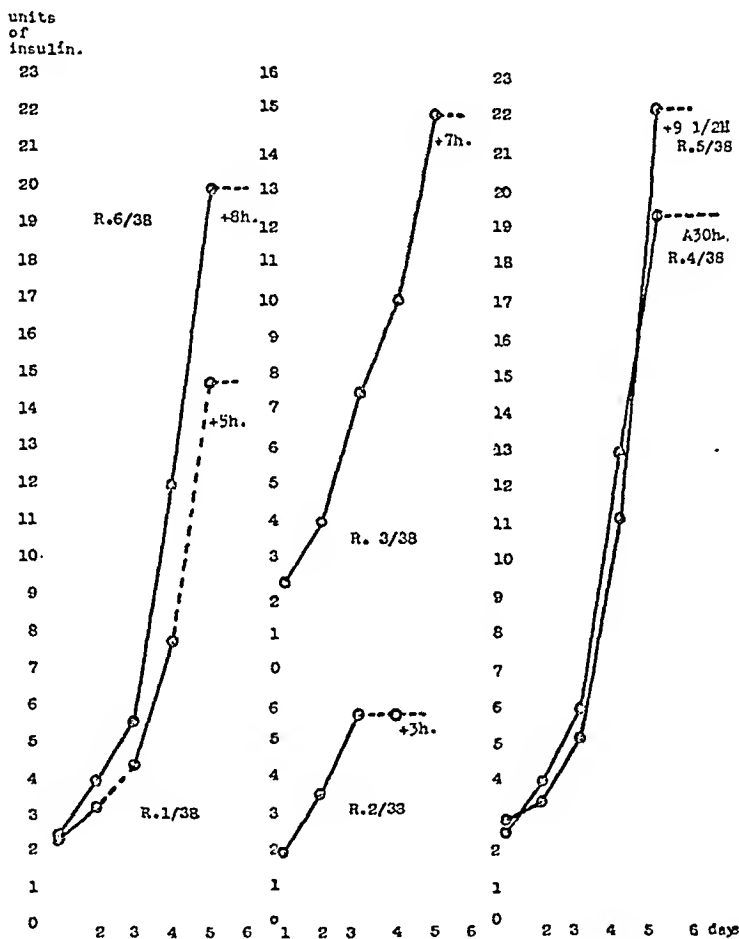


CHART 1. Effect of insulin (units per kilo body weight) on the rabbits of Series I. The broken lines indicate the days on which convulsions were observed.

+ = indicates spontaneous death; A = sacrificed by air embolism; N = sacrificed by suffocation in pure nitrogen. The figure followed by "h" indicates the period of time between the last injection of insulin and death in hours.

and on which days convulsions were elicited. This can be explained only to a slight extent by the fact that the rabbits were not fasted prior to the experiment. Marked muscular weakness and pronounced listlessness and apathy were present, many

times being the sole symptoms even after the administration of large doses of insulin. In some instances the muscular weakness progressed to paralysis of the musculature of the extremities and the neck. Violent convulsions which began suddenly, or after the animal was slightly touched, were noted as a rule from the 3rd to the 6th hour after the administration of an appropriate dose of

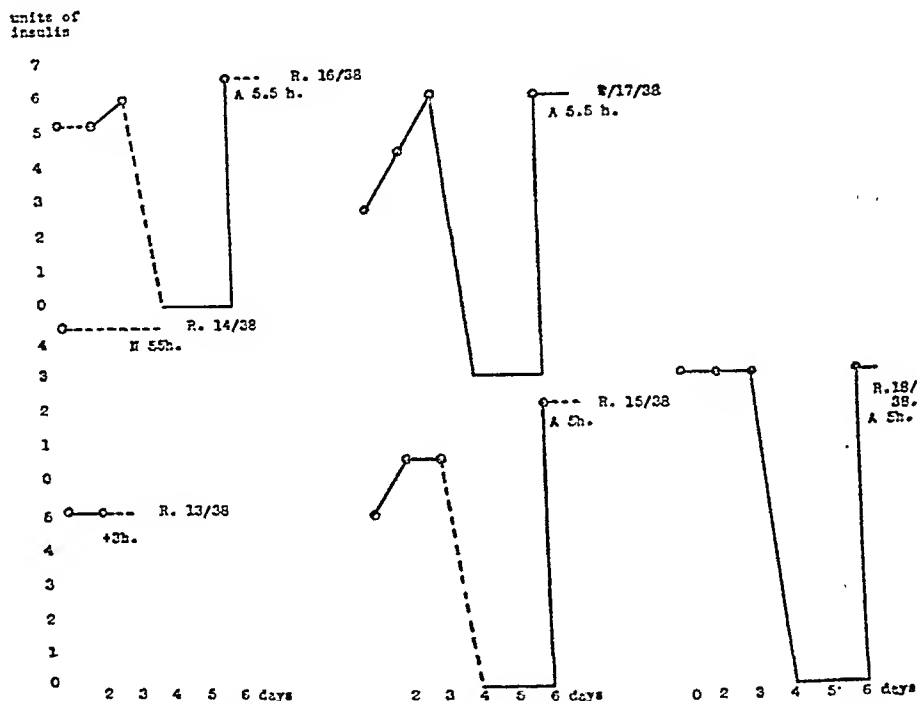


CHART 2. Effect of insulin (units per kilo body weight) on the rabbits of Series II. The broken lines indicate the days on which convulsions were observed.

+ = indicates spontaneous death; A = sacrificed by air embolism; N = sacrificed by suffocation in pure nitrogen. The figure followed by "h" indicates the period of time between the last injection of insulin and death in hours.

insulin; in a few instances they were apparent in the 2nd hour. Some rabbits failed to have convulsions in spite of very high doses of insulin given on several consecutive days; others were seized by most violent convulsions up to entire exhaustion following the administration of comparatively small doses. A spontaneous gradual recovery from the comatose or semicomatose state took place from the 6th or the 7th hour after the injection of insulin, even when convulsions were present, provided the insulin doses were

not excessively high for the particular rabbit, as they were on the 5th day for the rabbits of the 1st series.

All rabbits of the 1st series, except Rabbit 4, succumbed under large doses of about 20 units of insulin administered on the 5th

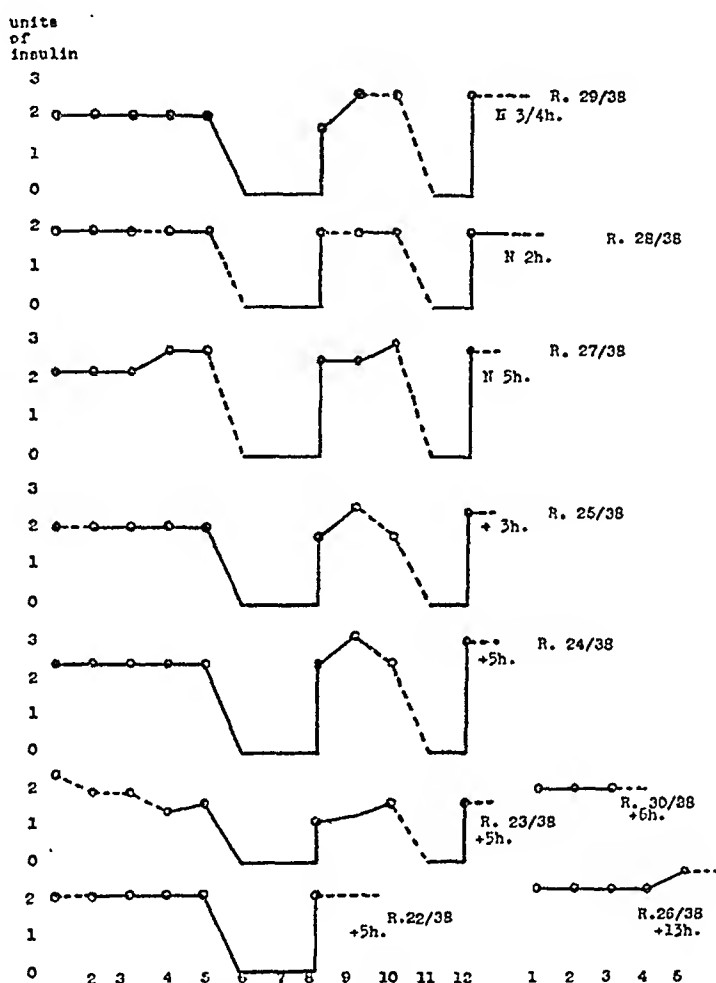


CHART 3. Effect of insulin (units per kilo body weight) on the rabbits of Series III. The broken lines indicate the days on which convulsions were observed.

+ = indicates spontaneous death; A = sacrificed by air embolism; N = sacrificed by suffocation in pure nitrogen. The figure followed by "h" indicates the period of time that elapsed between the last injection of insulin and death in hours.

day. They died between the 6th and the 12th hour following the insulin injection in spite of administration of glucose. Only Rabbit 4 survived when it was given glucose intravenously 9 hours after the insulin injection. It had been in deep coma with intermittent convulsions for more than 3 hours. Further subcutaneous

injections of glucose 3 hours later and on the next morning failed, however, to arouse it from the weak semicomatose condition in which it was, lying flat on its stomach, until it was sacrificed by air embolism 30 hours after the last injection of insulin.

Another remarkable reaction was shown by Rabbit 2 of the 1st series. On the 3rd day of treatment with insulin,  $6\frac{1}{2}$  hours following the injection of 5.8 units, it was seized by such violent convulsions that the respiration ceased. It was resuscitated by an intravenous injection of 10 cc. of 40 per cent glucose 1 minute later when the color of the blood in the ear artery had already turned deep blue. On the next day, however, the same dose of insulin was fatal after 3 hours, in spite of glucose given intravenously immediately after cessation of the respiration.

The great discrepancy between doses of insulin administered and reactions displayed was especially apparent in the 2nd series of animals. The 2 rabbits that were given the highest doses, from 4.4 to 8 and 9 units, repeatedly on consecutive days (Rabbits 17 and 18) failed entirely to have convulsions. In contrast, the initial dose of 4.4 units rendered Rabbit 14 deeply comatose 4 hours after the administration, and provoked frequent and violent convulsions during the next  $1\frac{1}{2}$  hours until glucose was given subcutaneously. This was, however, too late, and in spite of maintaining the blood sugar level above 100 mg. per cent by further injections of glucose, the rabbit was unable to raise its head or move its forelegs during the next 24 hours. Its condition became slowly worse so that after 48 hours the hind legs also were paralyzed. In that condition it was killed by nitrogen 55 hours after the 1st injection of insulin. Especially strange was the reaction of Rabbit 13 of this series. An initial dose of 5.1 units produced only slight listlessness on the 1st day. The blood sugar corresponded well to this slight reaction. It dropped from 106 mg. per cent to 56 mg. per cent  $4\frac{1}{2}$  hours after the insulin injection and rose spontaneously again to 113 mg. per cent 3 hours later. The rabbit was fed on the evening of the same day and was given glucose subcutaneously. On the next morning, however, the same dose of insulin was fatal after 4 hours.

In the 3rd series of rabbits in which the insulin doses were kept between 2 and 3 units, coma was not artificially interrupted by glucose except in Rabbit 23. In this rabbit the initial dose of 2.4

units was lowered to 1.2 and 1.6 units on the following days. Deep coma was usually terminated in this series by spontaneous recovery after a duration of about 3 hours. Rabbit 23, however, had to be given glucose subcutaneously on 4 days when its condition was so weak that spontaneous recovery could not be expected. The initial dose of 2.4 units, comparatively small for a non-fasting rabbit, had produced deep coma and convulsions from the 4th to the 7th hour after the injection. By this time when glucose was administered subcutaneously the rabbit had already suffered irreparable injuries to motor areas of the brain, for on the next morning it exhibited peculiar wiggling movements of its head and neck which never disappeared during the next 12 days.

### RESPONSE OF THE BLOOD SUGAR

The blood sugar level usually reached the lowest point in the 3rd hour after administration of insulin, then spontaneously rose again more or less rapidly. On the day when high doses of from 14.8 to 22.2 units were administered to the rabbits of the 1st series, the lowest blood sugar values were from 2.2 to 17.9 mg. per cent in the 3rd hour, even then rising spontaneously to from 25 to 37.5 mg. per cent in the 6th hour and from 45 to 71 mg. per cent in the 9th hour.

The height of the blood sugar level found in the rabbits corresponded well with the symptomatology exhibited. This, however, does not mean that the rabbits were always found in a state of deep coma or convulsions when the blood sugar level was at the lowest point. It was found that the blood sugar had to remain for some time at a low level before convulsions started. This level was about 40 mg. per cent. It was found that the incidence of convulsions depended much more on the failure of the organism to raise the blood sugar spontaneously again above a critical threshold within about 1 hour than on the deep fall itself. Thus, in the 1st series convulsions were not observed in the period when the blood sugar had almost entirely vanished, but in a period more than 1 hour later when it failed to rise again above 40 mg. per cent. On the other hand the rabbits, 17 and 18 for instance, that failed to have convulsions in spite of repeated injections of 8 and 9 units of insulin maintained a comparatively high blood sugar level. It was found to be 56 and 71 mg. per cent respec-



tively in these rabbits during the 4th hour after the injection of similar doses of insulin.

### CHANGES IN THE HEART MUSCULATURE

Cross sections through the hearts of the rabbits of the first 3 series at close inspection showed a peculiarly mottled or checkered appearance of the wall especially distinct at the posterior wall of the left ventricle. Tiny dots, fine streaks and small flecks, pinpoint to pin-head in size and gray in color, were recognizable there. The gross picture was somewhat suggestive of fatty infiltration although the color of the flecks was a bit too gray. Specific stains for fat with sudan III, scarlet red and Nile blue sulfate, however, were entirely negative and ruled out this suggestion. A peculiar hydropic swelling of the musculature was revealed histologically. In the areas involved the muscle cells stained much paler with hematoxylin-eosin than in normal areas. Individual muscle cells were swollen and enlarged, the striations were indistinct, and here and there had entirely vanished. The muscle cells appeared to be almost homogeneous and hyaline. The papillary muscles of the left ventricle, and occasionally of the right ventricle also, presented a similar appearance. In most of the hydropic muscle cells the nuclei were visible but were enlarged and pale. Swelling of the muscle cells was occasionally so marked that the blood capillaries were entirely compressed. In other areas of the same heart the capillaries of such hydropic foci were filled to capacity with erythrocytes giving the appearance of an artificially injected heart. This appearance, which indicated the capillaries had been incapable of constriction at the moment of death, was suggestive of stasis or severe local functional circulatory disturbance just prior to death. There was, however, no evidence that stasis might have been developed a considerable period of time (perhaps 1 hour or more) prior to death. In such an instance some massing of leukocytes would have been expected in the adjoining capillaries; this however, was not observed.

The glycogen stain (Best's method) was negative in these hydropic areas. In all the hearts of rabbits of the 4 series examined only very slight traces of glycogen, if any at all, were found histologically except in the cells of the conductive system which had preserved a glycogen content morphologically well demonstrable.

The hydropic foci in the musculature were the principal and constant finding in the hearts of practically all rabbits of the first 3 series, whether they died spontaneously following entire exhaustion or were sacrificed at various periods of the insulin shock by air embolism or suffocation in nitrogen. The size of the areas involved and the severity of the changes, however, varied considerably in the 3 series and in the single animals of a particular series. While in most animals the normal structure of the swollen muscle cells was preserved, in some instances small or even larger vacuoles were present in certain muscle cells. This was observed in Rabbit 1 of the 1st series, for instance, which on the 5th day of the experiment died spontaneously  $5\frac{1}{2}$  hours after the administration of 14.8 units of insulin. An autopsy was performed 15 minutes after death.

The hydropic changes in general appeared to be of quite recent formation, as if produced during the last exposure to an insulin shock. In the majority of cases there was no reactive cellular proliferation and no other sign indicative of previous cell destruction. Only 4 rabbits (1 and 2 of the 1st, and 22 and 23 of the 3rd series) showed evidence of irreversible damage to a few muscle cells of the many involved in hydropic changes. These cells had lost their nuclei, and their hyaline appearing and at times shrunken cytoplasm was invaded by scavenger cells, while a slight round cell infiltration was present in the interstitial spaces.

It was essential to rule out the fact that the method of sacrificing the rabbits was responsible for the formation of the above described hydropic changes. This was improbable from the beginning, for the rabbits that died spontaneously showed the same changes as those killed by air embolism or suffocation in a nitrogen chamber. The suffocation had to be given specific consideration, since the same changes could be produced by repeated anoxic shocks, as has been reported elsewhere. Five rabbits of the 4th series, which were sacrificed 7 weeks after the termination of the insulin treatment, served as controls in this respect. After they were placed in a nitrogen chamber, respiration and action of the heart ceased within 6 to 9 minutes. During this period the heart had been working under the strain of rapidly increasing anoxemia until it failed. But morphologically there was no trace of a hydropic swelling and vacuolization of cardiac musculature was

not observed. This indicates that a longer period is required for the development of these morphologically demonstrable changes. Many control rabbits sacrificed by air embolism after being used for various other purposes also failed to show these changes. On the other hand a single insulin shock of the duration of 4 to 5 hours was sufficient to produce these changes. Rabbit 14 is an example. The hydropic changes in its heart were very definite and widespread when it was sacrificed 55 hours later. Swelling and slight multiplication of the cells of the capillary walls in the areas involved made it doubtful whether or not the hydropic changes had surpassed the state of entire reversibility in this instance. Rabbit 13 showed definite hydropic changes but there was no evidence of irreversible damage. This rabbit died about 4 hours following the 2nd administration of 5.1 units of insulin. On the 1st day it was apparently not severely affected by that dose. The rabbits of the 3rd series which had been given daily doses not exceeding 3 units showed the same hydropic changes when they died or were killed from 2 to 5 hours after the last injection of insulin. Finally, Rabbit 33 of the 4th series, which 5 hours after the injection of a single dose of 0.82 units of insulin was exposed for 30 minutes to an anoxic shock and killed at the end of it, exhibited definite hydropic changes in the heart. The blood sugar dropped to 49 mg. per cent 2 hours after the insulin injection, and rose again to 69 mg. per cent immediately before the beginning of the anoxic shock.

In summary, it may be said that hydropic changes in the heart can be produced by a single dose of insulin large enough to produce a comatose or semicomatose condition for several hours. Convulsions were no prerequisite as shown by Rabbits 17 and 18, for instance, which had never had convulsions but exhibited quite definite hydropic changes in the heart.

Histological study of the hydropic changes in a single case did not permit a clear decision as to their reversibility, but this was possible by reviewing and comparing the entire series of animals. If the hydropic changes were the morphological equivalent of beginning irreversible cell destruction, evidence of fully developed necrosis, reactive cellular proliferation or scarring should have been found in the hearts of the rabbits that were repeatedly exposed to severe insulin shock, as were the rabbits of the 2nd and 3rd series. This, however, was not the case; in only the few

mentioned exceptions was there evidence of such a development. Even then, only a few cells of a comparatively large hydropic area were definitely necrotized and invaded by scavenger cells. Neither was there evidence of irreversible damage in the muscle cells that contained large vacuoles. These failed to give a positive reaction for fat or glycogen.

Of all the rabbits that were treated with smaller doses than those of the 1st series, only 22 and 23 of the 3rd series exhibited cellular reactive changes in the hydropic areas of the heart. They were the rabbits that had been most sensitive to insulin, as they were seized by violent convulsions after the administration of about 2 units of insulin on the 1st day of the experiment. They finally died in the 5th and 6th hour after the insulin injection on the 9th and 12th day, respectively, when the treatment was continued. In the heart of Rabbit 23 small foci of round cell infiltration were found centered around a necrotized muscle cell which was invaded by scavenger cells. This arrangement permitted their distinction from foci of chronic myocarditis which are rather common in the hearts of rabbits (Miller<sup>8</sup>). Fresh foci of hydropic swelling were also present. In Rabbit 22 there was found an entirely hyalinized area in the large papillary muscle bordering an especially severely involved hydropic area.

The absence of such changes in the majority of the rabbits showed that the hydropic changes on the whole can be regarded as entirely reversible. The complete absence of chronic inflammation or scarring at the usual site of the hydropic changes in the rabbits of the 4th series which were sacrificed after a period of recovery of 7 weeks strongly confirms this view.

As to the period of time required for the full restoration of the hydropic cardiac muscle cells, definite figures cannot be given. It may only be noted that Rabbit 36 of the 4th series showed scarcely a remnant of such changes when it was sacrificed by suffocation in pure nitrogen 2 days after the last insulin administration and 3 days after the last anoxic shock.

#### CHANGES IN THE SKELETAL MUSCULATURE

Gross examination of the skeletal musculature was not suggestive of severe changes. There were no gross disruptions in any case, not even in the rabbits that had had the most violent convulsions for hours. Portions of the iliopsoas muscle, the muscu-

lature of the thigh, abdominal wall and diaphragm were routinely removed at autopsy and studied. Histologically, however, quite marked changes were found, producing a checkered or mottled appearance of the stained muscle sections, somewhat similar to the hydropic changes described in the cardiac musculature. Many fibers stained lightly, were swollen, and had partly lost their striations. In many rabbits of the first 3 series still more definite changes were present also, especially in the iliopsoas muscle. They consisted of partial or entire disruption of single muscle fibers. They were found with variable frequency at times in each field of low microscopic magnification; in other instances one or two broken fibers only were present in a whole section. Not infrequently a muscle fiber was broken into many short segments which were held in their position by the sarcolemma only. Here and there merely an infarction of a muscle cell had occurred; the reactive cellular infiltration, however, gave evidence of the intravital occurrence of the injury. All transitional stages from the most acute disruption to healing and organization of the necrotized segments were observed. In fresh material, as in Rabbit 33, which died 5½ hours after the 1st injection of insulin, the broken ends of a torn fiber were retracted, swollen, of a hyaline or wax-like appearance and were pale staining; a few erythrocytes were present at the ragged ends of the disrupted fibers. Larger hemorrhages, however, were not found in such areas. Even in fresh disruptions a massing of muscular nuclei was often noted near the ends of torn fibers. These, apparently, had been mechanically dislodged. At a later stage a slight infiltration of the broken fibers by leukocytes, and later by round cells was observed. In instances such as Rabbit 23, which had been seized by severe convulsions on the 1st day but had been kept alive 12 days, definite calcified segments of broken muscle fibers were found. In some instances single muscle cells definitely shrunken and infiltrated by round cells were found. Obviously an excessive hydropic swelling alone had been able to necrotize these cells.

In all probability the violent convulsions observed played a major part in these muscular changes. Slight fibrillar muscular twitchings that escaped the attention might also be a sufficient mechanical factor.

As to the glycogen content of these muscles, it can be stated

only that they were morphologically free of glycogen. Chemical tests were not performed.

### CHANGES IN THE LIVER

In the majority of the rabbits of the first 3 series the liver was definitely changed in gross appearance. It was somewhat enlarged, peculiarly stiff, and firmer in consistence than normal but not doughy, as in a fatty liver. The color was more grayish than normal and cross sections were frequently abnormally dry. On close examination with the naked eye a bright central area of the lobules could be distinguished from the darker, more brownish red periphery. The gross appearance was somewhat suggestive of central fatty infiltration although no fatty film could be observed on the knife used for making the cuts. Aside from these diffuse changes, in a few cases, as in Rabbits 4 and 14, irregularly distributed grayish foci, pin-point to pin-head in size, were found. Their whole appearance was quite suggestive of focal necrosis.

Histologically by specific staining the suggestion of fatty infiltration was entirely ruled out. Only in Rabbit 26 was a marked fatty infiltration of the liver found, but only in the periphery and not in the center of the lobules. The marked difference between periphery and center of the lobules, recognized in gross was, however, found histologically also. In contrast with a passively congested liver, the capillaries in the centers of the lobules were markedly narrower than at the periphery, as though compressed by the large and swollen hepatic cell strands present. A photograph of the liver of Rabbit 30 which died spontaneously may serve as illustration.

The main factor responsible for the pale staining of the centers of the lobules with the hematoxylin-eosin stain was the changed histological structure of the hepatic cells, which were enlarged, swollen and extremely vacuolized, frequently to such a degree that the entire cell seemed to be emptied. The nuclei of most of these definitely changed cells were comparatively little involved. They were in the center of the cell, were not pushed to the periphery or sickle-shaped, as in severe fatty infiltration, and usually preserved their normal density. Only relatively few cells had lost their nuclei or exhibited a shrunken or pyknotic appearance. The pale centers showed usually quite a sudden transition to the better

stained and more compact cell strands in the periphery. The pale appearance of the central cells was simply produced by a spreading out of the cellular cytoplasm over a wider area because of intracellular storage of a material that did not stain with hematoxylin.

In the first 2 series the pale areas of the lobules were usually confined to the central third or half of the lobule. In the 3rd series the pale areas extended more toward the periphery, and comprised almost the entire lobule with the exception of a small rim around the branches of the portal vein. Best's glycogen stain revealed that these vacuolized cells were laden to full capacity with glycogen, while the small compact cells at the periphery were morphologically entirely depleted.

The livers of the 5 rabbits of the 4th series which had been sacrificed by suffocation in nitrogen 7 weeks after termination of the insulin treatment, and the livers of 5 normal rabbits which were sacrificed by blows on the neck served as controls. In accordance with the findings of Arndt <sup>4</sup> and Villaret and coworkers <sup>5</sup> the control rabbits showed a rather even distribution of a moderate amount of glycogen throughout the entire lobules of the liver. In the livers of the rabbits killed by suffocation the glycogen was slightly diminished in the peripheral parts of the lobules.

Compared with these control rabbits the main difference found in the livers of the rabbits of the first 3 insulin series was the peculiar distribution of the glycogen present. In sharp contrast with the entire depletion of cells at the peripheries of the lobules, there was a definite overload of glycogen in the cells of the centers of the lobules. Based on histological estimation this overload was so marked that the total amount of glycogen in the liver appeared to be larger than in the control rabbits.

Even the rabbits of the 1st series, which on the last day had been given extremely high doses of insulin, still showed considerable glycogen in the centers of the liver lobules, in spite of violent seizures lasting for hours, and death from complete exhaustion. This was found even when the autopsy was delayed up to more than 1 hour after death. Only Rabbit 5 was an exception. It was given the highest dose of insulin, 22.2 units per kilo body weight. The blood sugar dropped to 17.9 mg. per cent 2 hours later, and was found to be 29.5 mg. per cent in a sample of blood withdrawn directly after the first seizure, 5½ hours after the injection of

insulin. After 4 hours of intermittent convulsions 5.2 gm. of glucose were injected intravenously and subcutaneously in 20 per cent solution. This terminated the muscular twitching within 30 minutes but could not save the rabbit. It was autopsied 2 hours after death. The entire glycogen content of the liver was depleted and the liver presented histologically the appearance of acute passive congestion, in contrast with the usual findings. The hydropic changes in the heart of this rabbit were especially marked in the walls of both ventricles.

Rabbits 3 and 6 of the 1st series which had not been given glucose on the last day of the experiment, although they were comatose for hours, also showed a marked storage of glycogen in the centers of the lobules of the liver when they died  $7\frac{1}{2}$  and  $8\frac{1}{2}$  hours, respectively, following injection of insulin.

The 4 rabbits (15, 16, 17 and 18) of the 2nd series constitute an interesting group. Their blood sugar content was determined immediately before they were sacrificed by air embolism on the 6th day of the experiment when they were treated with doses ranging from 6.6 to 9 units. Rabbits 15 and 16 had had convulsions on preceding days; Rabbits 17 and 18, however, had none. They were sacrificed about 5 hours following the last insulin injection. Rabbit 15 had just had the first convulsion on this day. The blood sugar level then was 22 mg. per cent, but the liver cells in the central half of the lobules were overladen with glycogen, the capillaries being constricted. The blood sugar of Rabbit 16 was 20 mg. per cent. It was weak and comatose, but had had no convulsions on the 6th day of the experiment. In the liver glycogen was present only in the central third of the lobules, the single cells containing not more than normally found throughout the lobule. The capillaries in the center were still more constricted than those at the periphery. Rabbits 17 and 18 were somewhat listless but by no means exhausted when they were sacrificed. Their blood sugars of 49 and 59 mg. per cent respectively corresponded well to their general response. Their livers resembled closely those of normal rabbits, the capillaries having about the same width throughout the lobule and some glycogen being present in all cells, somewhat more in the liver of Rabbit 17 than in that of 18.

Comparison of these 4 rabbits indicates that those able to re-



lease the glycogen storage in the liver gradually into the blood were able to maintain a fair blood sugar level and avoid convulsions, in spite of high doses of insulin. The other rabbits, in whose livers considerable amounts of glycogen were locked up, developed severe symptoms although their insulin doses were not so high.

The livers of all rabbits of the 3rd series, with the exception of Rabbits 26 and 30, were laden with an amount of glycogen scarcely ever found under normal conditions, although several of these rabbits died spontaneously of exhaustion during a shock period.

Rabbit 26 was found dead in the 13th hour after the injection of 2.6 units of insulin on the 5th day. It had had convulsions on this day beginning 2 hours after the injection of insulin and continuing intermittently for more than 5 hours. On the 4 preceding days its response to 2.2 units of insulin had been only weakness and listlessness with spontaneous recovery after several hours. During these days its blood sugar level did not drop below 60 mg. per cent during the 2nd and 3rd hour after the injection of insulin. Its liver showed a typical peripheral fatty infiltration, being histologically entirely depleted of glycogen. The capillaries were wider in the centers than at the peripheries of the lobules. This was the only rabbit that showed a fatty infiltration of the liver.

Rabbit 30, treated for 3 days with 2 units of insulin, did not show severe symptoms on the first 2 days, but on the 3rd day seizures began in the 5th hour after the injection of insulin and were repeated several times up to its death at the end of the 6th hour. On that day the blood sugar was 41 mg. per cent at the end of the 1st hour after the injection of insulin, and did not rise to more than 51 mg. per cent  $3\frac{1}{2}$  hours after the first seizure. The liver was histologically depleted of glycogen although it presented the reverse picture of a passively congested liver.

Obviously all carbohydrates available in the organisms of these 2 rabbits had been used up during days of treatment with insulin. This might be the reason that on the last day convulsions began comparatively early and the blood sugar failed to rise spontaneously again. It was all the more striking that the other 7 rabbits of this series which were treated with similar doses exhibited so much glycogen in the liver.

Thus, Rabbit 22 died on the 9th day of the experiment, 5 hours

after the injection of 2 units of insulin. The same dose had produced convulsions on the preceding day, from which it had recovered spontaneously. Although the autopsy was not performed until 1 hour after death, the entire liver was quite overladen with glycogen.

Rabbit 23 had been the most sensitive of all the animals to insulin. Doses of 1.6 to 2.4 units had produced severe coma and seizures during 12 days. Its blood sugar would fall to a level between 22 and 33 mg. per cent during the period from the 2nd to the 5th hour after the injection of insulin. When it finally died, 5½ hours after the last injection of 1.6 units of insulin, its liver, removed 20 minutes after death, was heavily laden with glycogen. The changes in the vital organs, such as the heart and the brain, were in contrast exceptionally severe, practically all ganglion cells of the brain cortex, for instance, having disappeared.

Additional evidence that the livers of rabbits may store and lock up large amounts of glycogen under the influence or, more cautiously speaking, after the administration of insulin, while the whole organism may perish for lack of glycogen, is shown by the next group of rabbits to be described.

Rabbit 25 died spontaneously on the 12th day of the experiment exactly 3 hours after the injection of 2.5 units of insulin. When the respiration ceased no attempt at artificial respiration was made. A blood sample withdrawn from the hepatic vein 10 minutes later had a sugar level of 476 mg. per cent, evidence that considerable glycogen available in the liver was converted into blood sugar after cessation of the respiration due to the effect of anoxemia. The blood sugar level before the insulin injection had been 116 mg. per cent on this day. It would fall to from 27 to 33 mg. per cent during the period from the 2nd to the 5th hour after the injection of the same dose of insulin on several previous days. Histologically this liver was also overladen with glycogen.

Rabbits 28 and 29 were sacrificed so that the glycogen content of the liver could be studied in the 1st hours after the injection of insulin. Rabbit 29 had had severe convulsions on the previous day, the 12th day of the experiment, but had recovered spontaneously. On the next morning its blood sugar was 168 mg. per cent. Forty minutes after the injection of 2.7 units of insulin it dropped to 79 mg. per cent. Immediately after the withdrawal of

this blood sample this rabbit was suffocated in a nitrogen chamber. Thirteen minutes after the onset of anoxemia a blood sample withdrawn from the hepatic vein contained 357 mg. per cent of sugar. The blood sugar of Rabbit 28 was similarly high, 155 mg. per cent, on the same morning. It dropped to 62 mg. per cent 2 hours after the injection of 2 units of insulin. A short convulsion of about 1 minute's duration occurred immediately after the withdrawal of this sample of blood. The rabbit was then also suffocated by nitrogen. Six minutes after the onset of fatal anoxemia a blood sample withdrawn from the hepatic vein contained 116 mg. per cent of sugar. Histologically the livers of these 2 rabbits were still overladen with glycogen, as shown by Figures 18 and 19.

That the rabbits of the 4th series which had been given doses of about 1 unit reacted in a similar way is shown by the following results. Two hours after a single dose of 0.8 units the blood sugar of Rabbit 33 dropped to 49 mg. per cent and increased again to 69 mg. per cent after 3 hours. The rabbit was then exposed for 30 minutes to an anoxic shock that was fatal. Fifteen minutes after death blood from the hepatic vein contained 500 mg. per cent of sugar. Histologically the entire lobules of the liver were markedly overladen with glycogen. Three other rabbits (31, 32 and 34) which were treated the same way, except that the anoxic shock was not fatal, showed the following movement of the blood sugar. It was, on the average, 63 mg. per cent 2 hours after the insulin injection, 99 mg. per cent at the 5 hour mark, and 159 mg. per cent  $\frac{1}{2}$  hour later after the termination of the anoxic shock.

In the central areas of the lobules that were definitely overladen with glycogen relatively little morphological evidence was found indicating irreversible damage to the hepatic cells. Only a few cells had lost the nuclei or exhibited a pyknotic appearance. It could therefore be expected that the liver cells on the whole might be able under proper conditions to rid themselves of the overload of glycogen and return to a normal state. However, in the animals that had been treated with excessively high doses of insulin, such as the rabbits of the 1st series on the last day, there was found definite cellular damage. These rabbits exhibited single cells or small groups of cells in the liver with an eosinophilic cytoplasm and shrunken, hyperchromatic or even pyknotic nuclei. It would have been difficult to differentiate this appearance from postmor-

tem changes if it had not been for the presence of definite areas of focal necrosis in the livers of half the rabbits of the 1st series. These areas of necrosis were usually located in the centers of the lobules extending more to the periphery than to the center, occupying a sixth to a third of a lobule. Judged by the leukocytic and cellular reaction in the vicinity, they apparently formed on the last day of the experiment when high doses of insulin were given. In Best's stain for glycogen the necrotic foci were seen to be depleted of glycogen, even when all neighboring cells were laden. In the 2nd series only Rabbit 14 showed focal necroses in the liver. In accord with its survival for 55 hours after the single insulin dose given, the leukocytic and cellular reaction in the vicinity was marked. Many bizarre, hyperchromatic giant nuclei were found in the liver of Rabbit 13 which died about 4 hours after the second injection of 5.1 units of insulin. In only 1 rabbit of the 3rd series (Rabbit 30) were small foci of quite recent necrosis found. In spite of relatively low doses of insulin the liver of the rabbit was entirely depleted of glycogen. The animal died after violent seizures 6 hours after the last injection of insulin.

In a few instances a slight round cell infiltration, non-leukocytic in character, was noted around the small branches of the portal vein of 1 rabbit of the 1st series, 2 of the 2nd, and 3 of the 3rd. None of the rabbits of the 4th series showed such changes. It is hard to decide whether or not these changes are sequellae of the insulin treatment. Chaikoff, Connor and Biskind<sup>6</sup> reported cirrhosis of the liver in dogs that had been kept alive for years by injections of insulin after extirpation of the pancreas. Possibly changes similar to these are forerunners of such a development.

## DISCUSSION

In the experiments reported here, changes in the brains of the rabbits were so marked and dominant that spontaneous death when recorded could be attributed to their occurrence. According to the studies of Himwich and coworkers<sup>7</sup> these changes can be attributed readily to starvation of the brain, which maintains its metabolism solely by combustion of carbohydrates. Our studies have shown that the heart and skeletal musculature also are severely affected when the blood sugar drops to and is maintained

at low levels for hours as in insulin shock. Müller<sup>8</sup> has described electrocardiographic changes in patients during insulin shock treatment for schizophrenia. These changes were similar to those known to be present in severe organic myocardial conditions, but were transient and disappeared after termination of the insulin shock. Salm<sup>9</sup> described attacks of cardiac weakness and failure in such patients. Even if these might be considered sequellae of damage suffered by circulatory centers in the medulla rather than direct effects of the low blood sugar level on the heart musculature, observations reported by Larsen<sup>10</sup> furnish evidence for such a direct effect. He found electrographic changes similar to those reported by Müller when he subjected diabetic patients who were merely under the influence of small therapeutic insulin doses to the anoxic test designed for the objective evaluation of the actual power of the heart. The same patients showed normal electrocardiograms during the anoxic test when examined without insulin injections.

The hydropic changes reported above in the heart musculature of rabbits treated with insulin can be regarded as the anatomical equivalent of these electrocardiographic changes. They are also reversible, on the whole, and only in a few exceptional cases did small muscular necroses occur.

Weakening of the heart by insulin treatment is shown by another observation also. Normal rabbits sacrificed by confinement in a nitrogen chamber died within 6 to 9 minutes. Their lungs collapsed when the thorax was opened. Rabbits that had been in insulin shock for a few hours, even when no convulsions occurred, developed a marked pulmonary edema with bloody foam at the mouth when sacrificed in the same way. A weakened action of the left ventricle was obviously its main cause. Goldblatt's<sup>11, 12</sup> observation that rabbits with insulin hypoglycemia died of strychnine convulsions surprisingly rapidly can be similarly interpreted.

In the skeletal musculature still more severe changes were found than those in the heart. They were equivalent to the early and marked muscular weakness the rabbits displayed during insulin shock. These changes were present even when no convulsions had occurred. Various authors have described the vanishing of the glycogen content of the muscle after the onset of insulin convulsions (Baur, Kuhn and Wacker,<sup>13</sup> Dudley and Marrian,<sup>14</sup> Hoet

and Marks<sup>15</sup>). Hetényi<sup>16</sup> and Peyer<sup>17</sup> found that when the glycogen in the cardiac muscle had dropped during insulin convulsions from 0.35 to 0.12 per cent, that of the skeletal muscle was 0.05 per cent and less.

The anatomical findings in the heart and skeletal muscle seem to indicate that the heart as well as the skeletal musculature cannot act in the living animal for periods extending over several hours without structural damage unless a certain high level of blood sugar is maintained. This conclusion seems not quite to concur with observations reported recently by Himwich and Fazekas.<sup>18</sup> They found that the oxygen consumption of muscle did not diminish like that of brain when the glucose concentration of the blood was lowered by insulin administration. This led them to the conclusion that muscle is able to maintain its energy metabolism solely by the combustion of fat and that carbohydrates are not essential. Our anatomical findings at least make it clear that muscle in an insulinized animal requires carbohydrates in addition to the oxidative metabolism which might be maintained solely by fat. Without venturing a suggestion concerning the phase of the complicated muscular metabolism for which glycogen is indispensable, it might be conjectured that at times, for short periods, during action the oxidative metabolism is unable to provide momentarily the energy required so that the muscle has to rely on sources of energy that are accessible anaerobically. In all probability it is this type of energy metabolism for which glycogen is required. This conclusion finds some confirmation in observations made by Bogue, Evans and Gregory.<sup>19</sup> They found that the storage of glycogen in the beating heart which was artificially perfused was exhausted in about 3 hours. A sudden failure occurred at the point of this exhaustion which could be prevented only by supplying glucose with the perfusing fluid. They also observed that a heart excised after its depletion of glycogen fell immediately into rigor mortis, *i.e.* underwent irreversible changes. Concurring with these observations are those of Bayliss, Müller and Starling.<sup>20</sup> They maintained the action of artificially perfused hearts for several hours longer when the percentage of glucose in the perfusing fluid was increased.

The changes found anatomically in the heart and musculature were unevenly distributed. This undoubtedly points to a vascular

influence. Under normal conditions it might be insignificant whether a muscle fiber gets its blood supply mainly from a capillary that arises directly from an arteriole, or from a net capillary that arises from another capillary. But when the nutritional qualities of the flowing blood are lowered as much as in insulin shock, this might become a factor of importance, especially when strenuous action of a muscle is required. Even the difference of anatomical relationship to the venous or arterial end of a capillary might be of weight under such conditions. By the close inter-relationship of capillary wall and tissue pathological metabolic products that are produced by a muscle fiber inadequately supplied are likely to affect the wall of the capillary and produce local circulatory disturbances up to entire stasis. This would open a vicious circle that might finally be the cause of irreversible cellular changes.

Another actual observation has to be considered in this connection. During insulin convulsions and often even 30 minutes after the termination of severe seizures, the ear arteries of the rabbits became definitely constricted, frequently so intensively that it was impossible to obtain blood from the ear veins even when xylol was applied locally. If such vascular constrictions also occur in the parenchymatous organs and in the musculature, they might definitely contribute to severe tissue damage.

On the basis of studies by Dudley and Marrian,<sup>14</sup> McCormick and Macleod,<sup>21</sup> Staub,<sup>22</sup> Bissinger, Lesser and Zipf,<sup>23</sup> Cori,<sup>24</sup> Barbour *et al.*,<sup>25</sup> and many others, it is generally accepted that in the normal animal the glycogen content of the liver is depleted by insulin given in doses that produce convulsions. In a survey of the literature Staub<sup>22</sup> stated that in normal animals it is impossible to obtain an increase in the glycogen content of the liver by insulin unless, as in fasted animals, a deficit of that hormone is brought just to an equilibrium. Any excess would produce a decrease.

There seems to be a definite discrepancy between these statements and the results obtained in our experiments. In order to understand it several factors have to be considered. First, the method of our experiment was not that usually employed, in so far as no fasted normal animals were employed which, following a single injection of insulin with simultaneous feeding of glucose, or without any feeding, were sacrificed after a short period of a few hours.

Our rabbits could be considered normal only at the time they received their 1st injection of insulin. The 2nd injection given 24 hours later was administered to a rabbit that was already in a state of recovery from a serious disturbance of its carbohydrate metabolism. Each subsequent injection of insulin may have been with a somewhat different constellation of the hormonal equilibrium and its effect on the organs it governed. Some evidence for this contention is given by the blood sugar level which in the 2nd week of the treatment of the 3rd series of rabbits was found 4 times above 150 mg. per cent in the morning prior to the administration of insulin. The different states in which the rabbits received insulin after the 1st day may therefore be answerable mainly for the differences in our results from those generally reported. Another reason may be that in our studies the staining method of Best was employed for ascertaining the glycogen content of the organs. This method does not show glycogen in small amounts as are chemically still demonstrable, but it shows the peculiarities of its distribution in the liver and the concomitant vascular effects that might be worth while following still further.

The results of the experiments reported here indicate that the fate of a rabbit exposed to insulin in shock dosage depends very much on the reaction of its liver. Two types of reaction were observed. In rabbits that endured insulin doses of 8 and 9 units per kilo body weight repeatedly administered without severe symptoms, and that maintained a blood sugar level above 50 mg. per cent, the liver contained only a slight amount of glycogen, almost evenly distributed with little preference for the centers of the lobules. The capillaries were about the same width throughout the lobule. Obviously such a liver was able to release all glycogen synthesized during the shock period as blood sugar, preventing severe starvation of the brain and convulsions thereby. In rabbits with this type of reaction a total exhaustion of glycogen present in the body might still occur. Thus, rabbits that had endured large insulin doses on the preceding days finally died, at times surprisingly, when the dose was only a little increased or just merely repeated. In such instances the liver was regularly found to be free of glycogen.

On the other hand, rabbits that had displayed severe symptoms—convulsions for hours following the administration of ever



comparatively small doses of insulin — and whose blood sugar had dropped to vanishing levels, showed a liver overladen with glycogen when they died or were sacrificed at various periods of the shock. The capillaries in the liver, especially in the central portions of the lobules, which were mainly overladen with glycogen, were markedly constricted, producing the impression that thereby the glycogen was locked up. The majority of the rabbits showed this type of liver.

The influence of the dose of insulin was demonstrable in so far as the glycogen storage in the livers of rabbits given doses between 2 and 3 units was usually larger than in those receiving 5 to 20 units per kilo body weight. In principle, however, both types of reaction were found in all series of rabbits.

Only a few experimental observations are reported in the literature which concur with the results reported above. Frank, Nothmann and Hartmann<sup>20</sup> found an increase in the glycogen content of the liver in fasting rabbits when small doses of insulin were given. The experiments most important in this respect are those of Goldblatt.<sup>11,12</sup> He used young rabbits (about 400 gm. in weight) which had been fasted 24 hours prior to the experiment. The glycogen content of the liver increased up to sevenfold when insulin in convulsion-producing doses was given, and it was not depleted when the rabbits finally died of exhaustion. The actual observations of Goldblatt were confirmed by Corkill.<sup>27</sup> Other species, such as mice, ferrets and chickens, however, did not accumulate glycogen in the liver under the same conditions. Corkill came to the conclusion that the increase of glycogen in the livers of rabbits was not an effect of insulin alone but a complicated response of the organism possibly produced by the reactive output of adrenalin. Cori<sup>24</sup> had shown that adrenalin is able to produce hyperglycemia without depletion of the glycogen in the liver. Goldblatt had found that adrenalin did not prevent the increase of glycogen in the livers of his insulin rabbits, and in specific experiments Corkill showed that adrenalin increased the glycogen in the liver about four to five times, as compared with the average content of fasted control rabbits. In Ricker's laboratory, Loeffler and Nordmann,<sup>28</sup> by direct microscopic observation of the liver, found after injection of insulin in shock dosage a definite vascular constriction present for 2 to 3

hours. During this period glycogen was stored in the livers of rabbits, while in rats under the same conditions no glycogen but fat appeared, accompanied by a still more extensive vascular constriction. Insulin administered when a large storage of glycogen in the liver had been produced by an appropriate diet had a dilatory effect on the blood vessels at the peripheries of the liver lobules, simultaneously depleting the glycogen in these areas while the vascular bed and the glycogen in the centers of the lobules were not affected.

We are inclined to consider the vascular constriction in the liver a secondary effect of the insulin injected, or due to hormones secreted by the adrenal gland and the anterior or posterior lobes of the pituitary which reactively might be poured out into the blood stream. Hormones from these glands have been shown in recent years<sup>29-31</sup> to be effective in directing the carbohydrate metabolism.

At this stage it seems appropriate to remark that insulin administered in doses high enough to produce an initial marked hypoglycemia initiates a sequence of disturbances of the carbohydrate metabolism during which, obviously, hormones of antagonistic effects are liberated. These play their part in the carbohydrate metabolism, and produce vascular disturbances also.

#### SUMMARY AND CONCLUSIONS

Insulin in shock doses repeatedly administered to rabbits produced definite hydropic changes in the cardiac and skeletal musculature even when no convulsions were elicited. The posterior wall of the left ventricle and its papillary muscle were the most favored locations. The changes in the heart, on the whole, were reversible; only in exceptional cases did they progress to necrosis of small patches of cardiac muscle cells. In the skeletal musculature, especially in the iliopsoas and the thigh musculature, irreversible changes were more frequent. They were present as broken single muscle fibers in all stages of the process beginning with fresh breaks up to entire healing or calcification.

These changes are considered evidence of the necessity of carbohydrates for the working muscles in the body. They are in all probability indispensable for certain phases of the anaerobic metabolism.

The sensitivity of the individual rabbit to insulin varied widely. It depended rather on the response of the liver than on the dosages employed.

Two types of liver reaction to insulin were observed widely independent of the size of the doses. (1) The liver was able to release gradually all glycogen synthesized before and after the insulin administration from various sources (absorption from the intestines, lactic acid from the musculature). In such instances, which constituted a small minority, a comparatively fair blood sugar level was maintained and convulsions and severe damage to vital organs, such as the brain and the heart, were avoided for relatively long periods of time in spite of repeated administration of high doses of insulin on consecutive days. The rabbits died finally when all glycogen available was exhausted.

(2) The liver retained the carbohydrate available as glycogen and locked it up, particularly in the centers of the lobules which were definitely overladen. This response was accompanied by a marked constriction of the capillaries in the overladen areas of the lobules. Thus, while large amounts of glycogen were stored in the liver, the blood sugar dropped to vanishing levels and convulsions with severe damage to the brain and heart occurred. The rabbits that were especially sensitive to insulin showed this result in the liver. The influence of dosage on the type of reaction in the liver was insignificant when doses between 2 and 20 units per kilo body weight were administered, the smaller doses producing merely further extension of the glycogen-storing area toward the peripheries of the lobules.

The glycogen storage in the liver is considered to be the effect of insulin and various other hormones of antagonistic or synergistic effect on the carbohydrate metabolism produced by the adrenal gland and the anterior and posterior lobes of the pituitary. Simultaneous vascular effects of these hormones are considered essential to produce such changes.

Aside from the reactions described, small areas of focal necrosis in the liver were observed, especially in the series of rabbits treated with excessively high doses of insulin.

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## DESCRIPTION OF PLATES

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### PLATE 6

- FIG. 1. Section from the heart of Rabbit 1 showing a focus of edema around an artery with round cell infiltration.
- FIG. 2. Section from another area of the heart of Rabbit 1 showing swelling and acute hydropic degeneration of the cardiac muscle cells.
- FIG. 3. Higher power of section from the same heart shown in Figure 2.
- FIG. 4. Heart from Rabbit 23. Necrosis of single cardiac muscle cells with round cell infiltration is seen.



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4

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Changes in Rabbits Treated with Insulin



PLATE 7

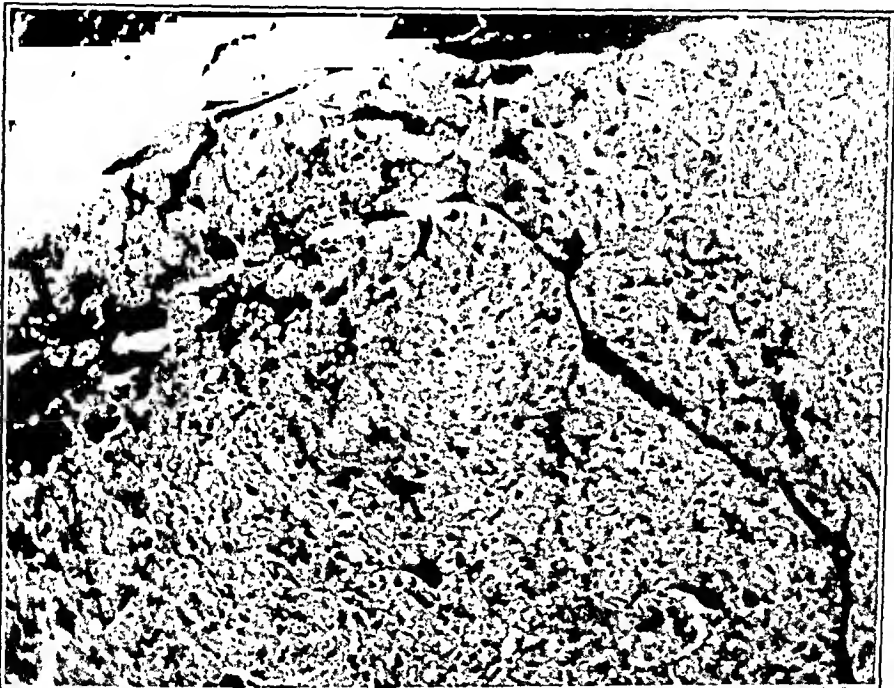
FIG. 5. Section from the right ventricle of the heart of Rabbit 5 showing marked hydropic swelling of the inner layers of the ventricular wall.

FIG. 6. Section from the left ventricle of the same heart showing similar changes.

FIG. 7. Iliopsoas muscle from Rabbit 15. Breaks in single muscle fibers and reactive cellular proliferation is seen. For higher power see Figure 10.



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Changes in Rabbits Treated with Insulin

PLATE 8

FIG. 8. Heart from Rabbit 22 showing definite hydropic swelling and hyaline degeneration.

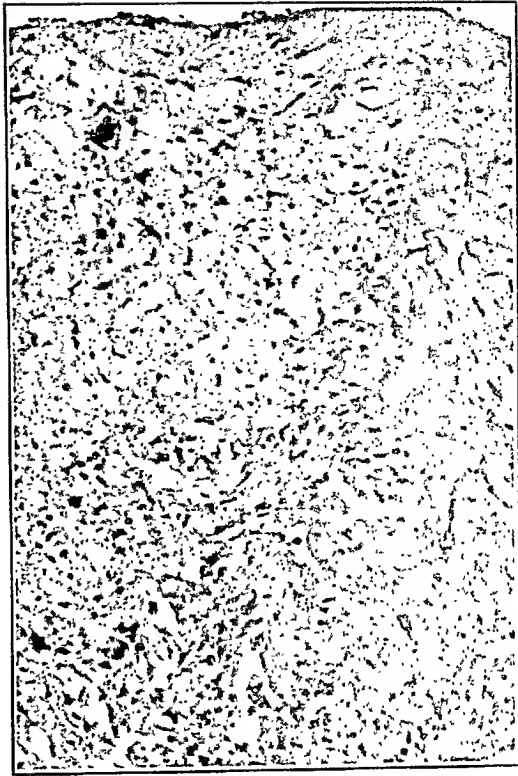
FIG. 9. Another area from the heart of Rabbit 22 showing hydropic swelling and hyaline degeneration.

FIG. 10. Higher power of field from the iliopsoas muscle of Rabbit 15 shown in Figure 7.

FIG. 11. Section from the liver of Rabbit 18 stained with Best's method for glycogen. Note the slight amount of glycogen present and the dilated sinusoids.



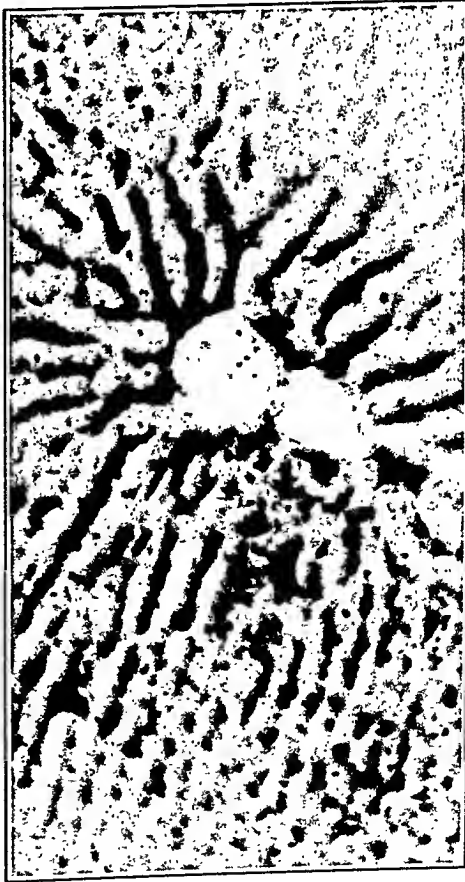
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Changes in Rabbits Treated with Insulin

PLATE 9

FIG. 12. Liver from Rabbit 1 stained with Best's glycogen method and showing a definite storage of glycogen in the central third of a lobule with depletion at the periphery. This rabbit was injected with 14.8 units of insulin on the last day of treatment.

FIG. 13. Higher power of section of the liver from Rabbit 1 shown in Figure 12 and stained by the same method.

FIG. 14. Liver from Rabbit 15 stained with Best's method for glycogen. A definite overload of glycogen is seen in the central halves of the lobules.

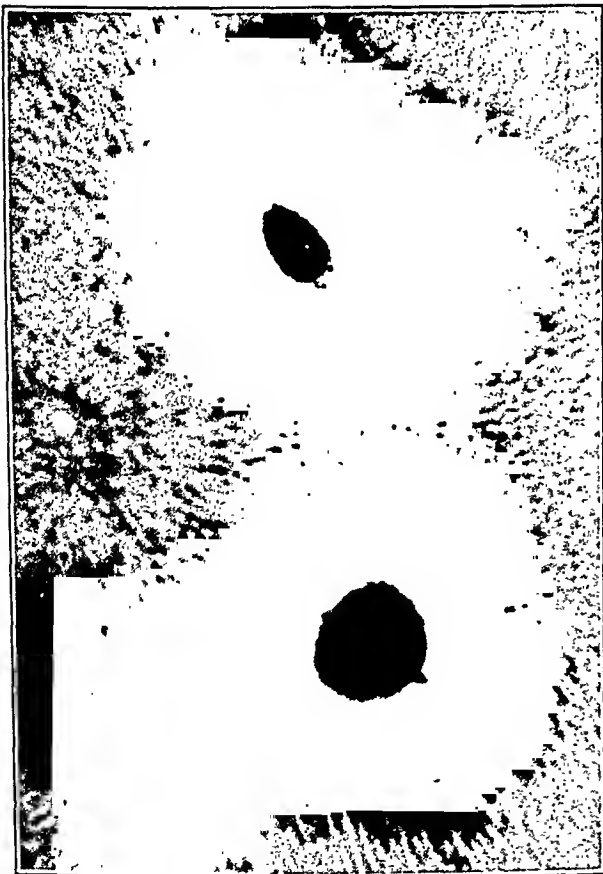
FIG. 15. Section of liver from Rabbit 23. The glycogen stain reveals a marked overload of glycogen in the entire lobule, especially in the center. This rabbit died from complete exhaustion after hours of convulsions.



12



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15

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Changes in Rabbits Treated with Insulin

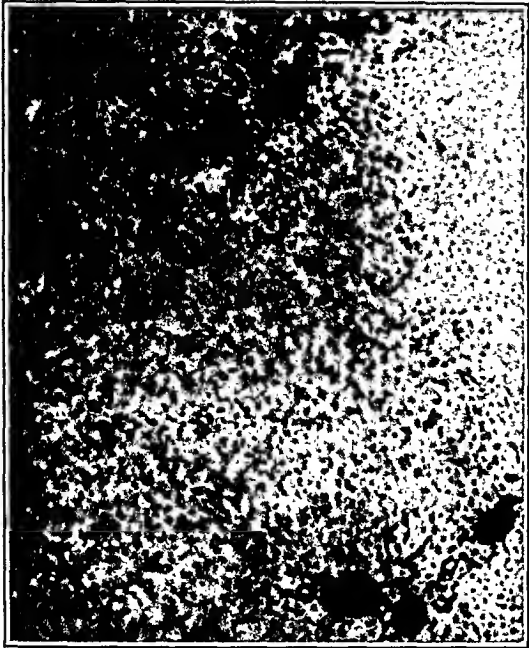
PLATE 10

FIG. 16. Liver from Rabbit 30. Note the marked congestion of the capillaries around the branches of the portal vein and in the periphery of the lobules, and the vascular constriction in the centers of the lobules.

FIG. 17. Higher power of the same liver shown in Figure 16.

FIG. 18. Section of liver from Rabbit 29. Best's stain for glycogen shows the definite overload of glycogen present, especially in the center of the lobule. The rabbit was killed 45 minutes after the last insulin injection.

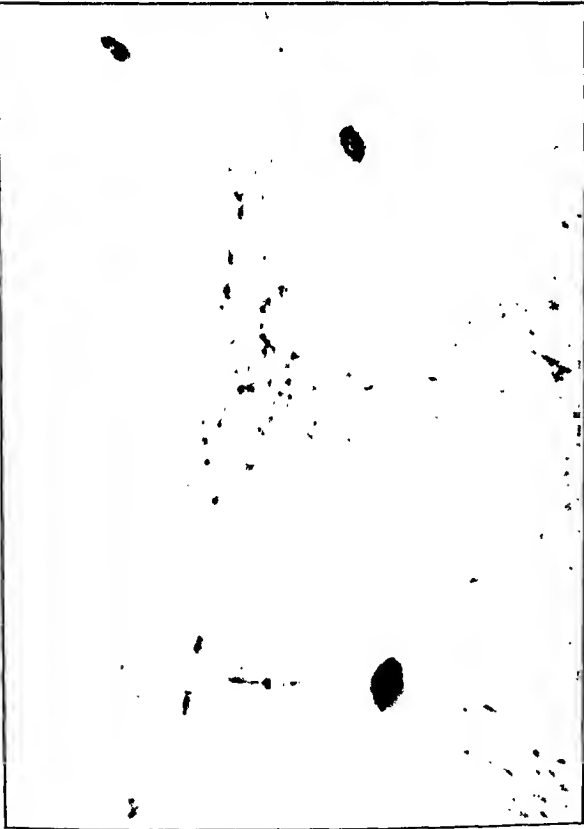
FIG. 19. Liver from Rabbit 28. The glycogen stain shows almost the entire lobule filled with glycogen. The rabbit was killed 2 hours after the last injection of insulin.



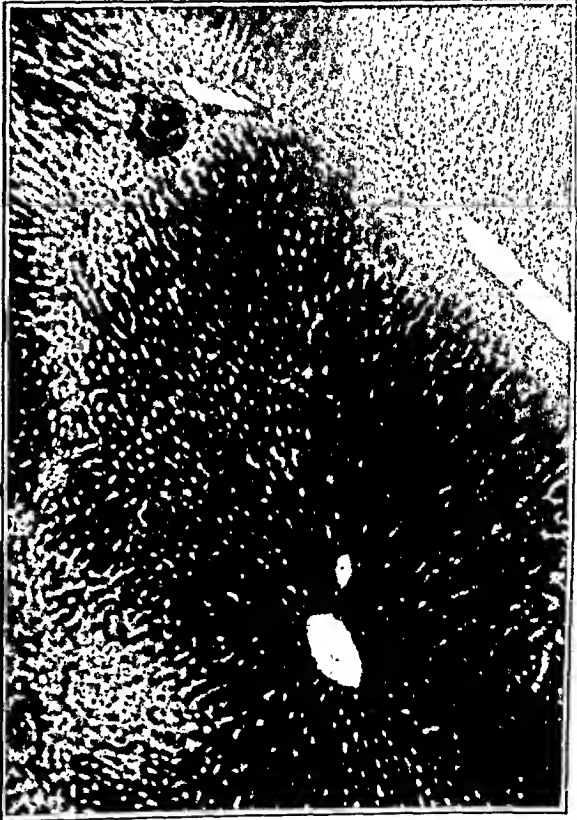
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Changes in Rabbits Treated with Insulin



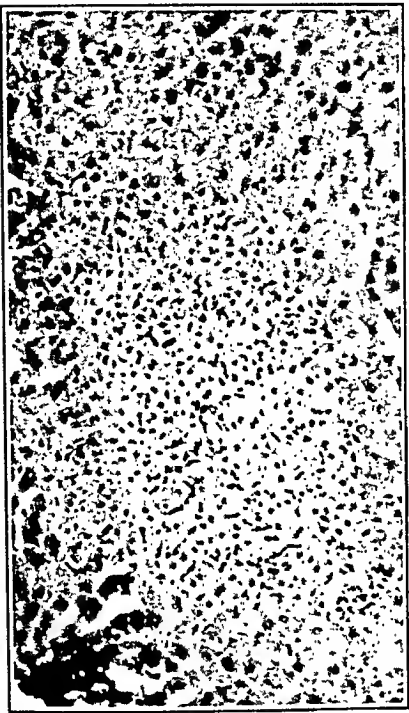
PLATE I I

FIG. 20. Liver from Rabbit 4 showing focal necrosis of quite recent origin.

FIG. 21. Section of liver from Rabbit 14 showing similar changes.

FIG. 22. Section of liver from Rabbit 3 stained by Best's glycogen method showing necrotic hepatic cells depleted of glycogen.

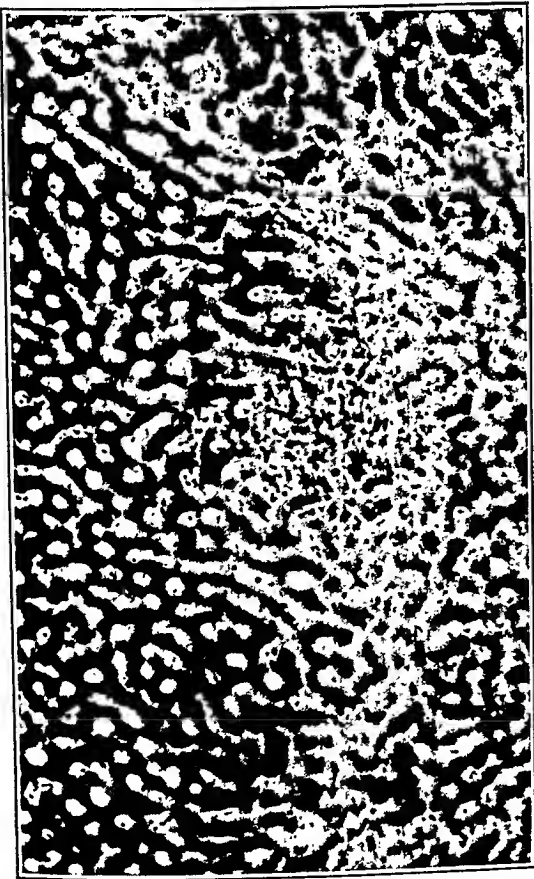
FIG. 23. Section of liver from Rabbit 6 showing single cells with pyknotic nuclei and eosinophilic cytoplasm.



20



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23

Changes in Rabbits Treated with Insulin



# GROWTH PROCESSES IN CARTILAGE AND BONE SUBSEQUENT TO GONADECTOMY AND ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT OF CATTLE IN IMMATURE MALE AND FEMALE GUINEA PIGS \*

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Differences in the body growth of male and female rodents under normal and pathological conditions have been established.<sup>1-3</sup> This and the fact that in man the disposition toward diseases of the osseous system varies in accordance with the sex suggest the possibility that differences might also exist in the response of the cartilage and bone-forming tissues of the two sexes toward stimuli of various kinds. In former investigations (1935)<sup>4</sup> we have shown a growth-promoting effect of acid extract of anterior pituitary of cattle on the cartilage of immature guinea pigs, regardless of their sex. Subsequently similar observations were made by Zuck<sup>5</sup> (1938) in guinea pigs, and by Freud and Levie<sup>6</sup> (1938) in rats. In continuation of our studies<sup>7</sup> we found an increased hyperplastic and hypertrophic growth of the epiphyseal cartilage after ovariectomy alone, and in combination with anterior pituitary extract. Since then we have extended our studies to the condition of the joints, bone marrow, cortex of the long bones, ribs and vertebrae in ovariectomized immature guinea pigs.

Recently we performed in addition experiments on immature male animals. In some, the influence of castration on the growth of cartilage and bone was studied, and in others the interaction of castration and anterior pituitary was investigated and compared with the results obtained in ovariectomized animals under the corresponding conditions.

## MATERIAL AND METHODS

In 43 male guinea pigs, born in the fall and winter, and weighing 135 to 160 gm., both testes were removed under ether anes-

\* These investigations have been carried out with the aid of a grant from the Committee on Scientific Research of the American Medical Association and a grant from the International Cancer Research Foundation.

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thesia by incision into the scrotum. Twenty animals did not receive any further treatment and were sacrificed after periods of 7, 9, 14, 21, 28 and 35 days. Fifteen guinea pigs received daily intraperitoneal injections of 1 cc. of freshly prepared acid extract of anterior pituitary of cattle. The injections were started immediately after castration. These animals were killed after periods of 7, 14, 21 and 28 days. In the third group, consisting of 8 castrated guinea pigs, the treatment with the extract was begun after 7, 14, 21 and 28 days respectively had elapsed following the operation. In these series, 7 injections were given and the animals were killed in groups of 2, 1 day after the last injection. Ten non-castrated animals of the same weight received a corresponding number of injections of acid extract, while 8 animals served as normal controls.

At autopsy the thyroid glands, adrenals, and in control animals one testicle also, were fixed in Petrunkevitch's solution and embedded in paraffin. The tibia from one side was taken out separately, while the knee joint of the other side was left intact by removing the tibia and femur as a whole. In addition, 2 to 4 ribs were removed in such a way that about 0.5 cm. of each cartilaginous and osseous part were preserved. Parts of the spine, comprising lower thoracic and upper lumbar vertebrae, were likewise removed. After the bones had become soft enough for cutting, the distal parts of the tibiae and the proximal ends of the femurs were cut off, and a sagittal section was made through the middle of the knee; the single tibia was cut in frontal sections. Thus, a study of the epiphyseal zone in two different planes was made possible. All bones were fixed, embedded and stained as described in previous papers.<sup>8</sup>

In addition, the joints, ribs and vertebrae of the 30 female animals which had served for the analysis of the endochondral ossification in ovariectomized guinea pigs<sup>7</sup> were examined. Altogether, the material of 91 guinea pigs was studied.

Although our investigations were not primarily concerned with the behavior of the weights of the animals under these experimental conditions, we thought it of interest to consider this factor also, and each animal was weighed three times a week.

In order to determine more definitely the rate of hyperplasia and hypertrophy of the cartilage cells of the epiphyseal line and

their mutual proportions, counts were made of the cells lying in certain rows. A mean was established by counting three rows in each section. Care was taken to choose layers in corresponding positions.

## OBSERVATIONS

The mean weights of the animals are represented in Tables I and II.

TABLE I  
*Male Guinea Pigs*

Experiment	Number of animals	Initial weight	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
		gm.	gm.	gm.	gm.	gm.	gm.
Normal	8	143	166	185	211	232	260
Injected	10	149	145	157	182	205	..
Castrated	28 *	147	173	200	226	254	315
Castrated and injected	15	149	147	162	194	220	..

\* This figure includes 20 castrated animals and 8 additional castrated animals in which the injections were started after 1, 2, 3 and 4 weeks following castration respectively.

TABLE II  
*Female Guinea Pigs*

Experiment	Number of animals	Initial weight	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
		gm.	gm.	gm.	gm.	gm.	gm.
Normal	13 *	140	165	195	213	242	272
Injected	13 *	143	149	157	173	197	238
Ovariectomized	19 *	145	165	195	230	252	280
Ovariectomized and injected	22 *	146	153	166	180	191	223

\* In these figures are included 37 animals from experiments which have been conducted since our previous communication.<sup>7</sup>

*Microscopic Examination**I. Normal Control Animals:*

In both sexes the finer structure of the cartilaginous and bony tissues is the same. No differences could be detected. A detailed description has been given previously by us.<sup>8</sup>

*II. Injected Control Animals:*

*Epiphyseal Line and Chondrophyte:* Seven to 28 injections of the extract, 1 injection being given daily, produce typical changes

which are essentially alike in both sexes. These changes have been discussed in previous reports.<sup>4</sup>

*Joints:* Arthropathic lesions<sup>9</sup> may be found in both sexes. Loops of capillaries of the bone marrow advance into the cartilaginous pressure zone eroding the thin bony lamella which usually separates the calcifying cartilage cells from the bone marrow. In the *female* hypertrophic changes of the cartilage predominate with a tendency toward degeneration. In the *male*, although hypertrophy may be present at later stages, hyperplasia is more accentuated.

The *bone marrow* adjacent to the zone of ossification assumes a fibrous character.

*Cortex:* The appositional growth and the resorptive processes are stimulated but well balanced. Osteoid seams cannot be detected.

*Ribs:* After 7 injections, in both sexes, but more markedly in males, a degeneration of the chondromucoid ground substance and of the mature euhyaline cartilage cells becomes noticeable (Fig. 1). The cell nuclei become pyknotic and lumpy, and the cytoplasm becomes enlarged, vacuolated and disintegrated. At the chondro-osseous junction the resting and columnar cartilage cells proliferate and hypertrophy. These changes increase with increasing numbers of injections of acid extract. They have to be considered as precursors of the "acromegalic rosary" which we have described previously.<sup>10</sup>

*Vertebrae:* The zone of endochondral ossification shows a hyperplastic and hypertrophic growth of the resting and columnar cartilage cells and an increased tendency to calcification. In addition, osseous plugs ("Ossificationsluecken," Erdheim,<sup>11</sup> and Schmorl<sup>12</sup>) may appear. These plugs consist of osseous masses within the zone of columnar cartilage and result from degenerations within the cartilage; they contain transverse cracks and irregular cavities. Even after 7 injections, small sized plugs may be observed, and they may be essential in counteracting a possibly widespread spontaneous fracture of the vertebrae softened by degenerative processes. The cracks indicate possibly that tension differentials in the tissues exist which otherwise would lead to fractures. It is a striking fact that these plugs are not found within the epiphyseal line of the long bones but only in the verte-

brae. Erdheim<sup>11</sup> explains this occurrence by differences in the gross structure of these two types of bones. In the long bones the epiphyseal disc is firmly fixed, supported and protected against breakdown by the overlapping bony cortex. This latter condition is lacking in the vertebrae and the necessary support of the bones is supplied by the osseous plugs.

### *III. Gonadectomized Animals:*

*Epiphyseal Line:* In *male* animals, 1 week after castration, the zone of endochondral ossification is of medium width and is patent. The chondromucoid ground substance which takes on a distinctly basophilic stain with hematoxylin is swollen, vacuolated and, in some cases, disintegrated. The network of the fibrils within the matrix is loosened and partly degenerated. Associated with these lesions are changes within the cartilage cells which may be summarized as follows: The cells of the resting and columnar cartilage become more numerous and the cytoplasm of the vesicular cartilage increases in size. Thus a typical hyperplasia and hypertrophy of the individual cells result. These phenomena were observed in all cases. In addition we detected occasionally very slight retrogressive changes within the cartilaginous cell rows similar to but less significant than those we reported after implantations of one-quarter of a gland of cattle anterior pituitary in young female guinea pigs on 2 consecutive days.<sup>8</sup> Increasingly from one week to another following castration the epiphyseal line becomes somewhat wider on account of an increased hyperplasia of the cartilage cells rather than because of an enhanced hypertrophy. The retrogressive changes within the intercartilaginous stroma are likewise augmented. After 4 to 5 weeks the epiphyseal line is very wide. At this stage degeneration of entire cartilaginous cell rows is seen. The cells of the hypertrophic calcifying cartilage zone are slightly increased in number and hypertrophic. At the periphery of these cells masses of calcium and lime salts have been deposited. The intercartilaginous matrix is scarce. The usually regular border line between the zone of calcifying cartilage and the bone marrow is preserved to a great extent. In some places, however, the breakdown and the replacement of the hypertrophic cartilage cells by bone have not at all, or only incompletely, been accomplished. Thus, in the majority of cases strands of densely



calcified hypertrophied cartilage are visible within the bone marrow. These thick strands which may contain as many as 5 or 6 or more cartilage cells show a longitudinal arrangement; the contours of trabeculae are already suggested. One has thus to deal with an increased calcification but incomplete ossification. The cartilaginous strands are accompanied by fibrous connective tissue and are surrounded by osteoblasts.

These findings are in contrast with the corresponding conditions in *ovariectomized* animals. In the latter, only a slight swelling and perhaps a trace of degeneration may be noticeable. There is an increased tendency to hypertrophy on the part of the cartilage

TABLE III

*Ratio of the Number of Hypertrophic Cartilage Cells to the Number of the Proliferating Columnar Cartilage Cells*

Experiment	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
Normal males and females	4-5:11	4:10	4:11	4:10-11	4:10
Castrated males	5:16	5:17	5:17-18	5:20	4:18-19
Castrated females	5:12	6:17-18	6:18-19	5-6:18	..

cells, while the rate of proliferation is about the same or slightly lower as compared with the males. On the whole, the epiphyseal line is wider in females than in males in the corresponding stages, the width in this case being determined by the degree of hypertrophy rather than that of hyperplasia. The tendency to calcification and ossification is less pronounced in the females. No strands of calcified cartilage within the bone marrow were observed, and ossification apparently proceeds at a well balanced pace, as indicated by a sharply demarcated regular boundary between the zone of calcification and the bone marrow.

A differential count of the number of cells in the columns of the proliferating cartilage, on the one hand, and the hypertrophic cartilage on the other, is given in Table III.

The data concerning the normal animals agree with the figures of Harris<sup>13</sup> who, in guinea pigs weighing 250 gm., noticed a normal ratio of 4:10 in the epiphyseal line of the upper tibia.

*Chondrophyte:* As early as 1 week following testectomy a

marked proliferation and hypertrophy of the euhyaline cartilage cells is seen. Consequently, typical basophilic "incubator" or "brooding" capsules of cartilage are produced. Because of the increase in number and size of cartilage cells, the intercartilaginous stroma is diminished in amount. It may reveal a slight tendency to basophilia. Toward the peripheral part an accelerated conversion of precartilaginous cell elements into mature cartilage cells takes place. These changes become even more accentuated at later stages following castration (Fig. 2). A fusion of hypertrophied cartilage cells may occur. Occasionally, multinucleated giant cell-like structures which may become dissolved are found, or, here and there, a degeneration of individual cartilaginous cells may take place. When the nuclei disintegrate the finer cellular structure disappears and the cytoplasm becomes vacuolated and lumpy, and shows irregular outlines.

As in the epiphyseal line, so also in the chondrophyte, the hyperplasia and degeneration of the cartilage cells are evidently more pronounced in *males* than in *females*. In the latter, extensive basophilic incubator capsules and degeneration have not been observed.

*Joints:* In *males* the cartilage cells of the surface of the joint are increased in number (Fig. 3 a). In quite a few areas 4 or more cells may be packed into one capsule. The acidophilic intercartilaginous ground substance is diminished in amount. The hyperplastic process affects primarily the cartilage cells of the transitional and pressure zone. This proliferation becomes marked about 2 to 3 weeks following castration. On the other hand, hypertrophic changes are only slight and, if they occur at all, usually occur at the earliest 3 to 4 weeks following castration. In addition, capillary loops of the bone marrow may protrude through the zone of ossification into the pressure zone and corrode the cartilage cells of the latter. These changes indicate the beginning of arthropathic lesions of a definite but not acromegalic type.

In *females*, 1 week following ovariectomy a hyperplasia of the cartilage cells within the various zones takes place accompanied or followed by a marked hypertrophy of the cells. At later stages (Fig. 3 b) a liquefaction of the hypertrophied cartilage cells may occur and give rise to lesions resembling in character the acromegalic arthropathia which we have described previously<sup>9</sup> as tak-

ing place after the administration of acid extract of anterior pituitary, although under the latter condition they are less marked than after castration.

Thus, the cartilage of the female joint shows in the first place a greater responsiveness than that of the male, and secondly a greater tendency to hypertrophy. This behavior agrees with that of the cartilage of the epiphyseal line in the two sexes.

*Bone Marrow:* The capillaries are congested. Strands of hypertrophic, incompletely ossified or non-ossified cartilage may be surrounded by osteoblasts laid down in ribbons along the outlines of these strands. They are accompanied by a more or less fibrous connective tissue. This condition is most pronounced 2 to 3 weeks subsequent to castration. The rather loose connective tissue may extend a greater or shorter distance into the metaphysis and toward the lymphoid marrow. Many mitoses of blood and connective tissue cells are seen everywhere in areas adjacent to the cartilaginous cell layers.

In *females* the marrow is likewise congested. The tendency to fibrosis is slight and is practically negligible. Strands of hypertrophied calcified cartilage were not seen.

*Cortex:* The periosteum and endosteum in both sexes are apparently intact. There is no evidence of a disturbance in the equilibrium between apposition and resorption of bone.

*Ribs:* In *males* the intercartilaginous stroma reveals retrogressive changes which increase with increasing intervals after castration. During the first 2 weeks swelling and degeneration predominate in the stroma, whereas during the 3rd week degeneration of the cartilage cells becomes noticeable, and this may reach a high degree during the 4th and 5th weeks (Fig. 4 a). The cells affected are found in the columnar cartilage of the chondro-osseous junction as well as in the resting cartilage of the cartilaginous part of the ribs.

In *females*, in accordance with what we observed in other cartilaginous tissues, the tendency toward degeneration is less marked. Hyperplasia of cells may occur but is less accentuated than in the males.

*Vertebrae:* The zone of endochondral ossification reveals conditions analogous to those seen within the epiphyseal line of the tibia. Degeneration as well as hyperplasia is more pronounced in

*males*, but hypertrophic changes are more frequently found in *females*.

#### IV. Gonadectomized and Injected Animals:

*Epiphyseal Line:* In *males*, as the number of injections increases the epiphyseal line of the tibia becomes progressively narrower than in castrated non-injected animals. The degenerative processes (Fig. 5) within the chondromucoid substance are evidently more accentuated and more extensive than in either castrated or injected control animals. A marked hyperplasia and hypertrophy of the cartilaginous cell elements becomes noticeable. However,

TABLE IV

*Ratio of the Number of Hypertrophic Cartilage Cells to the Number of Proliferating Columnar Cartilage Cells*

Experiment	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
Normal males and females	4-5:11	4:10-11	4:12	4:10-11	..
Testectomized and injected animals	5:16	4-5:17	5:18-19	6:18-19	..
Testectomy fol- lowed by 7 in- jections during the last week	..	6:16	5:16-17	3:15	3-4:14
Ovariectomized and injected animals	5:13	4:11	5:11	5-6:10	..

the hypertrophy does not exceed the degree obtained by castration alone. At later stages the pericellular calcium deposits cause a dense incrustation of the hypertrophic cartilage cells. Moreover, lines of deposited calcium are seen within the matrix of the columnar cartilage layer. But, even after 28 injections a complete calcification of the epiphyseal line, as found after the administration of the extract alone, was not observed. Differential counts of the number of proliferating and hypertrophic calcifying cartilage cells gave results as shown in Table IV.

The changes tabulated in Table IV are seen after a period of at least more than 7 days, during which a combination of castration and injections of extract was effected.

*Chondrophyte:* In *males* the hyperplastic and hypertrophic

growth as seen after either castration or injections of the extract alone may be intensified. Consequently, a number of incubator capsules degenerate, taking a dark blue stain with hematoxylin without revealing any structural details (Fig. 4 b). In the corresponding *females* the hypertrophic and degenerative changes are less pronounced.

*Joints:* In comparison with either castrated non-injected animals, or with non-castrated injected animals, the cartilage reveals a higher degree of response to the combined action of castration and acid extract. In *females* a marked hypertrophy is associated with a distinct hyperplasia. The cells of the sliding zone are not only increased in number, but the cells of the transitional layer take on a perpendicular arrangement, their cytoplasm at the same time increasing in size. Some cells may degenerate and liquefy, thus leading to the formation of minute cavities. The cells of the pressure zone are likewise hypertrophic. In addition, loops of capillaries and marrow cavities penetrate through the zone of ossification and invade the pressure and transitional zone, causing an increased resorption of the cartilage and corrosion of the surrounding bony lamellae. Thus, arthropathic lesions result.

In *males*, although hyperplastic growth occurs in the transitional layer, hypertrophic changes take place only after long treatment with the extract and never reach the same extent as in females. Consequently, arthropathic lesions are less frequently seen in males.

*Bone Marrow:* As in the case of castrated *males*, the bone marrow contains strands of hypertrophied calcified cartilage cells which are longitudinally arranged and suggest the shape of trabeculae, although ossification has not as yet or has only incompletely taken place. These strands may be surrounded by osteoblasts. The greater the number of injections given, the thicker, more cellular and massive are these strands. They are accompanied by a mass of fibrillar connective tissue which in some cases extends throughout the width of the subepiphyseal area of the bone marrow and is sharply demarcated from the normal metaphyseal bone marrow (Figs. 6 and 7). As a rule the maximum of fibrosis is reached after the 2nd week. At later stages the strands of cartilage and the fibrosis recede gradually.

As compared with the males, in *female* ovariectomized and in-

jected guinea pigs the cell proliferation is definitely decreased, the tendency to calcification and ossification is less accentuated, and the equilibrium between growth of cartilage and replacement by bone is well maintained. The border line between the zone of calcification and ossification is regular and only a few strands of calcified cartilage within the bone marrow were seen in 1 case.

*Cortex:* On the whole in both sexes the appositional and resorptive processes are stimulated but generally well balanced.

*Ribs:* The degenerative changes within the chondromucoid matrix as well as in the cartilage cells may be intensified as compared with those seen in either merely castrated or injected animals. A great number of cartilage cells are degenerated, revealing a disintegrated cytoplasm stained a deep blue with hematoxylin. As in the epiphysis of the tibia, wide areas are degenerated and entire cartilage cell rows may be affected. Furthermore, a distinct hyperplasia and hypertrophy of the resting and columnar cartilage cells are seen so that the stroma is diminished in amount. The marrow in the subepiphyseal layer is fibrous in nature. In 2 cases, in which following testectomy acid extract was injected over a period of 4 weeks, a broad ribbon of conglomerated hypertrophic cartilage cells was detected within the bony part of the rib, far away from the zone of ossification. At present it is difficult to understand why ossification failed to take place in this area. A fracture could be excluded in this instance.

Also, in the ribs the retrogressive lesions are more frequent in *males* than in the corresponding *females*.

*Vertebrae:* The behavior of the endochondral ossification in the vertebrae corresponds to that noted within the epiphyseal line of the tibia. Moreover, under the influence of the extract, in castrated and injected *male* guinea pigs, the occurrence of osseous plugs is a rather regular phenomenon (Fig. 8). In *females* they likewise occur but are much less frequent.

In the instances in which 7 injections were given after certain periods of time had elapsed since castration, the cartilaginous cells revealed an increased tendency to hypertrophic and degenerative changes in the chondrocyte and the joint. This increase was more notable the longer the interval between castration and the beginning of the injections. The columnar cartilage of the epiphyseal line showed a decreasing tendency to proliferation, whereas

hypertrophy was at first increased and then dropped below the normal level.

### COMMENT

Our investigations have shown that both injection of acid extracts of anterior pituitary of cattle and gonadectomy cause proliferative as well as retrogressive changes in the euhyaline cartilage of the immature guinea pig. These effects are not limited to the epiphyseal line of the long bones but extend to various parts of the skeleton. As stated above, in both sexes characteristic changes may take place in the epiphyseal line of long bones, joints, bone marrow, bony cortex, ribs and vertebrae. They consist of hyperplasia and hypertrophy of the cells as well as degeneration of cells and intercartilaginous matrix, increased calcification of the hypertrophic cartilage, and a tendency of the bone marrow to fibrosis.

The histological appearances vary and depend upon the degree in which the various tissues participate in these processes. This participation may be determined by hormonal stimulation, by factors inherent in the cells and tissues, or by both of these factors. Thus, under certain conditions one of the following changes may predominate: (1) proliferation of cartilage cells; (2) the process of calcification of hypertrophic cartilage; and (3) ossification of calcified cartilage cells.

(1) *Cell Proliferation*: This occurs in gonadectomy as well as after injection of small amounts of extracts and after implantation of anterior pituitary of cattle. This suggests that the growth-promoting effect of gonadectomy on the cartilage may be secondary to an increased production or liberation of the growth hormone of the anterior lobe of the pituitary gland. This might well be the case because it has been shown by Friedman and Loeb<sup>14</sup> that the stimulating effect of gonadectomy on the pituitary gland is a non-specific one, inasmuch as accompanying the increase in gonadotropic activity the thyrotropic action of the pituitary gland is also enhanced. The increased formation of cartilage cells similarly may be explained as being due to an increased growth-promoting activity of the anterior pituitary gland. Although following gonadectomy proliferation of the cartilage is activated in both sexes, it is the male in which the growth stimulation is more intense. This

would mean that the testicle exerts a stronger inhibition on the pituitary gland than does the ovary.

In this connection it is noteworthy that in gonadectomized male guinea pigs the proliferative activity of the thyroid gland is greater than in the corresponding females,<sup>15, 16</sup> an effect which may perhaps be due to an increased stimulation of this gland by the male pituitary gland after removal of the inhibiting action of the testicle. However, this interpretation is at present merely of a tentative nature.

(2) *Calcification*: In males the process of calcification of the hypertrophic cartilage is obviously more interfered with than in females, as evidenced by the appearance in the former of hypertrophied, heavily calcified cartilaginous strands. The question now arises whether calcification of the hypertrophic cartilage is also regulated by hormonal influences or whether it is due to nutritional disturbances, or to conditions inherent within the cells. Harris<sup>13</sup> contends that because of nutritional conditions a given mass of cartilage is not able to grow indefinitely but is subject to retrogressive changes as soon as a maximum size has been reached. In our experiments this is apparently true of the chondrophyte and the ribs, where a more or less extensive degeneration of matrix and cells occurs. However, conditions within the epiphyseal line seem to be different, and factors other than the maturation or senescence of the cartilage must be involved in the process of calcification and subsequent ossification. Under normal conditions a single cell row of the epiphyseal line consists of about 10 to 11 columnar, and 4 hypertrophic cartilage cells. Since calcification of the cartilage takes place at this level it should be expected that these numbers represent the maximum beyond which the cartilage cells cannot multiply without undergoing degeneration. This is not the case, however, in gonadectomized animals. In the gonadectomized female 6 hypertrophic cartilage cells are frequently found, while the number of cells in the proliferating columns averages 19. Furthermore, in such animals the epiphyseal line is much wider than in normal guinea pigs and its total volume is also considerably increased. Notwithstanding the increased volume, the senescence of the cartilage, as determined by its tendency to calcification, is not only not increased but on the contrary rather retarded as compared with the normal. This gain in the power of



resistance of the cartilage cells under these conditions may be due to changes in the action of certain hormones.

As to the process of calcification of the hypertrophic cartilage, it need not necessarily be associated with disturbances of the calcium level in the serum, but it seems to depend on changes in the tissues themselves which are induced by the hormones. But it is very unlikely that there exists a direct relation between calcification of the cartilage and the action of the pituitary gland in view of the fact that in ovariectomized animals the calcification of the cartilage is diminished in comparison with the normal, whereas in male castrated animals it is increased. If the pituitary should be involved one would expect the castrated female to show a degree of calcification intermediate between the normal and the male castrate. We must consider, however, the possibility that the thyroid or the sex glands are involved in this process. We have begun experiments to test this suggestion.

(3) *Ossification*: The mechanism of ossification is subject to factors similar to those active in the process of calcification of the cartilage; hormonal influences as well as local environmental conditions must be considered. Ossification depends on the action of certain constituents of the bone marrow on the calcified cartilage. However, in the course of our experiments it was possible under certain conditions to observe a partial dissociation between the process of calcification and ossification of the cartilage. A partial dissociation occurred when cartilaginous strands underwent hypertrophy and calcification but ossified only very incompletely. Such an insufficiency of ossification may result either from an overproduction of calcified cartilage or from a relative or absolute inability of the bone marrow to penetrate into the calcified cartilage and cause a breakdown of the cartilage cells. This condition is usually combined with a tendency to fibrosis of the bone marrow.

We have not as yet discussed the significance of *degeneration* within the epiphyseal line of male castrates. It might be tempting to correlate it likewise with the overproduction of cartilage cells. There are, however, certain considerations which render such a correlation improbable. In the first place we have observed similar degenerations in female guinea pigs, which had received 2 to 4 implants of one-quarter of a gland of anterior pituitary of cattle, and in which the number of the columnar cartilage cells was not

as high as in the case of castration of males at later dates. Moreover, if nutritional disturbances had been the cause of these degenerative changes, one would have expected the degenerated areas to be located in places farthest remote from the nutritional vessels, namely in the zone of calcifying cartilage and extending horizontally through the epiphyseal line (lines of arrested growth, Harris<sup>13</sup>). On the contrary, we find the areas of degeneration extending in a vertical direction all through the epiphyseal line parallel to the columns of cartilage. We may then assume that these degenerations, similar to those following implants of anterior pituitary, are due to a direct or indirect effect of the pituitary gland. Perhaps this condition can be tentatively explained in the same way as the calcification in males, namely as being due to the direct or indirect effect of the increased action of the anterior pituitary gland.

#### SUMMARY

In immature guinea pigs gonadectomy causes an increased proliferation of the euhyaline cartilage in various cartilaginous tissues. In males hyperplastic growth is more accentuated, whereas in females a more pronounced tendency to hypertrophy is present. The latter is especially evident within the joint, where arthropathic lesions are initiated more readily than in males.

In males degeneration occurs more frequently and to a greater extent than in females. Calcification and ossification of the cartilage appear balanced in females, but in males marked disturbances are observed in the zone of ossification. Associated with these disturbances is a tendency to fibrosis of the bone marrow.

The cause of the increased proliferation is seen in a non-specific stimulation of the anterior pituitary subsequent to gonadectomy. Differences in the behavior of the sexes are tentatively explained as due to differences in the inhibiting action of the gonads on the pituitary gland in males and females, and also to differences in the action of gonadectomy on the thyroid gland in the two sexes.

A combination of the action of acid extract of anterior pituitary of cattle and of castration leads to a summation effect which is determined by different degrees of stimulation exerted in the various tissues.

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## DESCRIPTION OF PLATES

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### PLATE 12

FIG. 1. Cartilaginous area of a rib from a male guinea pig which received 8 injections of acid extract of anterior pituitary of cattle. Numerous, deeply staining degenerated cells which do not show any structural details are seen.

FIG. 2. Male guinea pig 3 weeks after testectomy showing hyperplasia and hypertrophy of the chondrocyte with incubator capsules. Hyperplasia and hypertrophy are present in the epiphyseal line.



I



2

Growth Processes in Cartilage and Bone

Silberberg and Silberberg

### PLATE 13

- FIG. 3 a. Cartilage of the knee joint from a male guinea pig 4 weeks after testectomy. Marked hyperplasia but less hypertrophy is present than in the corresponding female (b).
- FIG. 3 b. Knee joint of a female guinea pig 4 weeks after ovariectomy. Marked hyperplasia and hypertrophy of the cartilage cells of the various zones is seen. Corrosion of the basal pressure zone is produced by protruding capillary loops of the bone marrow.
- FIG. 4 a. Male guinea pig 5 weeks after testectomy. Degeneration of the cartilaginous matrix is present with destruction of cartilage cells near the chondro-osseous junction in the rib.
- FIG. 4 b. Male guinea pig after castration and 12 injections of acid extract. Degenerated brooding capsules are present in the chondrophyte.



3 a



3 b



4 a

Silberberg and Silberberg



4 b

Growth Processes in Cartilage and Bone



PLATE 14

FIG. 5. Male guinea pig after testectomy followed by 12 injections of acid extract of anterior pituitary of cattle. Degeneration of whole cell rows and proliferation of the cartilage cells of the epiphyseal line is seen.

FIG. 6. Male guinea pig after testectomy and 12 injections of acid extract of anterior pituitary of cattle. Calcified and incompletely ossified strands of hypertrophic cartilage are seen protruding into the bone marrow of the tibia. Note the lack of lymphoid marrow between the strands. The epiphyseal line is narrow.



5

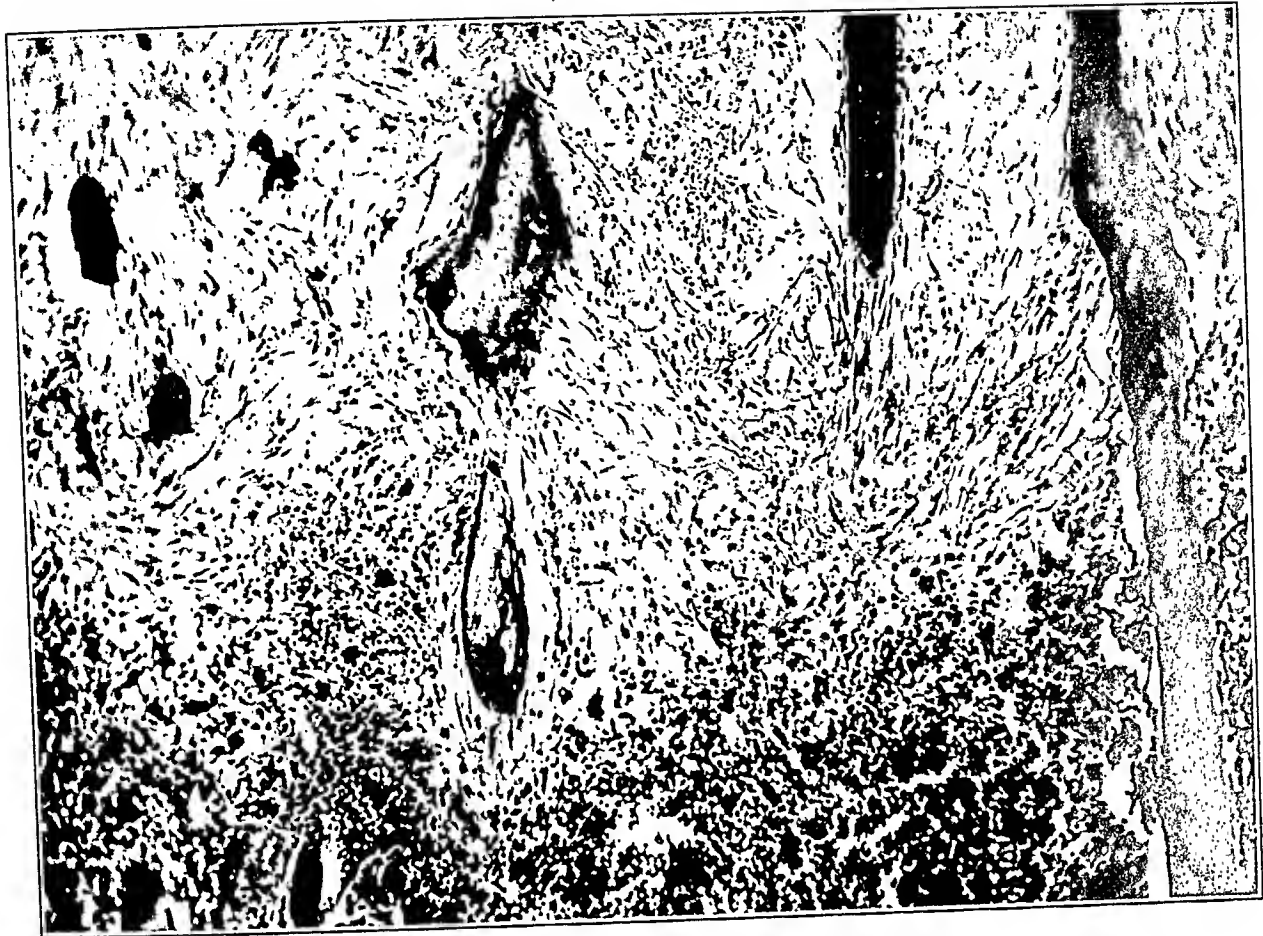


6

PLATE 15

FIG. 7. From the same guinea pig as shown in Fig. 6. Note the fibrosis of the bone marrow of the tibia. The lower part of the picture shows preserved lymphoid marrow.

FIG. 8. Male guinea pig after castration and 20 injections of acid extract of anterior pituitary of cattle. Section of vertebra showing hypertrophic and hyperplastic growth within the zone of ossification. Note the osseous plug with transverse cracks and two cavities in the center of the picture.



7



8



### III. THE DISTRIBUTION OF LESIONS AND ITS POSSIBLE SIGNIFICANCE

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In 1853 Virchow<sup>1</sup> described the distribution of the lesions in colitis as follows †: "In particular it is the projections of the mucous membrane, caused by the anatomical distribution of the muscle tissue, which are affected by preference. Hence the especial susceptibility of the mucosa along the insertion of the three longitudinal muscle bands, and of the transverse folds of membrane. In that the diphtheritic process establishes itself first on the projecting and later on the intermediate mucosa, there arise those regular geographical figures which indicate the intensity and mode of spread of the lesion."

Virchow attributed this peculiar distribution of the ulcerations in colitis to the closer contact of the fecal current with the mucous membrane in places where the lesions were most pronounced. Although this interpretation is scarcely tenable, his description of the ulcerations according to a muscular pattern is most accurate. Pathologists in general have overlooked this statement by Virchow and no one has ventured to explain the arrangement of the lesions in ulcerative colitis. It is the purpose of this paper to reconsider the location of the ulcerations in this disease and to advance a possible explanation for their occurrence.

During a period of 18 months 6 cases of ulcerative colitis came to autopsy at the Mallory Institute of the Boston City Hospital. Two of these patients had been ill with their first attack of ulcerative colitis only 2 months before death. Four had had previous attacks and died during an acute exacerbation of the disease. Cultures of stools and agglutination reactions for dysentery organisms were negative on five patients. On one, cultures of

\* Received for publication June 25, 1938.

† Translated by Flexner and Sweet.<sup>2</sup>

stools for pathogenic bacteria were repeatedly negative but *B. enteritidis* was recovered from the blood stream at autopsy.

In this group of cases the disease varied in its extent. One individual showed involvement of the rectum and sigmoid only; in three the lesions extended to the splenic flexure; in one to the midportion of the transverse colon; and one showed involvement of the entire colon without extension beyond the ileocecal valve. In all instances the most severe lesions were in the rectum, the walls of which were greatly thickened and indurated. The mucosa of the rectum was irregularly ulcerated. At the junction of the rectum and sigmoid the ulcerations developed into three rows running in the longitudinal direction of the intestine. Between these rows of ulcers the mucosa was edematous and indurated, and showed small erosions. In places there were ulcerations extending between the longitudinal rows of ulcers, and where this occurred they seemed to follow a linear pattern in the circular direction of the intestine. The bases of the ulcers were made up of the circular muscle fibers, and in most instances there was an undermining of the epithelial edges.

When the fat and mesenteric attachments were dissected away from the muscular layers of the intestine where this linear arrangement of ulcers was noted it was found that the lesions lay directly over the tenial bands. This correlation was found in all 6 cases. A possible explanation for the failure to note this distribution of ulcers previously is the manner in which the colon is usually opened at postmortem examination. Unless the wall between two tenial bands is opened deliberately, the teniae will frequently be transected in an irregular manner, thus obscuring the linear pattern of the ulcers.

Figures 1 and 2 show photographs of specimens taken from 2 cases of chronic, non-specific ulcerative colitis. The linear character of the ulcerative processes in the longitudinal direction of the intestine is clearly shown. Beneath each of these rows of ulcerations lies a tenial band. Figure 3 shows a low power microphotograph of another specimen in which a cross section of the colon reveals the manner in which the ulcerative process overlies the tenial band.

As mentioned previously, the intestinal tract was unevenly involved by the disease and lesions were found in all stages of

development in the same individual. The earliest changes noted in gross consisted essentially of hyperemia, edema and petechial submucous hemorrhages. In certain areas where the lesions had advanced to a later stage, superficial erosions occurred which were covered with fibrin. Other lesions were found which were interpreted as transitional stages between the earliest erosions and the frank ulcerations. A peculiar feature of the sections taken from these early lesions was the complete or almost complete absence of mucus in the epithelial cells. This finding was noted in all sections taken from the acutely involved areas and contrasted sharply with the appearance of the mucous membrane in a normal colon. Figure 4 shows a section of normal human colon with great numbers of goblet cells. Figure 5 shows for comparison a section from a patient who died from acute ulcerative colitis. The absence of mucus-containing goblet cells is quite striking.

### DISCUSSION

The peculiar pattern of the lesions in ulcerative colitis cannot be a mere accident, and an interpretation of their location may throw light on the etiology of this disease. The rectum possesses the most powerful muscles in the colon.<sup>3</sup> It differs from the rest of the colon in that it has no teniae and the longitudinal fibers are evenly distributed about its circumference. Above the level of the rectum the tenial bands are the most powerful part of the colonic musculature.<sup>4, 5</sup> The arrangement of the severest lesions in ulcerative colitis is such that they lie directly over the most powerful muscles, and there may well be a direct relation between the muscles and the lesions.

In this connection it is interesting to note that the early stage of ulcerative colitis can be simulated experimentally in animals by hypermotility and spasm of the colonic muscles. Elsewhere<sup>6</sup> one of us has reported experiments on explants of the colon in dogs. These explants were subjected to severe muscular spasm by the use of prostigmin intravenously and acetylcholine salt locally. The changes produced by these methods consisted first of diffuse hyperemia and edema. Within a few minutes petechial hemorrhages were noted beneath the surface epithelium, and later superficial erosions with hemorrhage oozing from the surface and marked induration of the explant appeared.



The microscopic appearance of biopsied material taken from the explants after muscular hyperactivity was found to be essentially that of a diffuse inflammatory reaction in the mucosa. In addition it was noted that whenever the explant underwent severe muscular spasm all of the evident mucus in the goblet cells had disappeared. From these experiments it seems reasonable to conclude that if the colon is stimulated to intense muscular activity, it is capable of producing severe damage to the mucosa, similar to the pathological changes noted in early ulcerative colitis.

Spasm and hypermotility of the intestinal muscles are a prominent clinical feature of ulcerative colitis. They are manifested by diarrhea, tenesmus and urgency, by the pipe-stem colon on X-ray examination, and by the contracted segment of lower intestine on sigmoidoscopy, which distends only with difficulty or not at all under positive pressure. It seems highly probable in view of these considerations that ulcerative colitis is primarily a disease caused by intense muscular spasm and hypermotility, and therefore the distribution of lesions follows a muscular pattern. Whatever infectious element is present may well be due to secondary involvement of the damaged areas caused by muscular overactivity.

#### SUMMARY AND CONCLUSIONS

In 6 cases of ulcerative colitis it was demonstrated at autopsy that the severest lesions were in the rectum and overlying the tenial bands. This distribution of lesions over the most powerful muscles of the colon may well be due to the fact that hypermotility and spasm of the colonic muscles cause the disease. Supportive evidence for this interpretation is the fact that early lesions of ulcerative colitis have been produced in the colons of dogs by hypermotility and spasm of the muscles alone.

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## DESCRIPTION OF PLATES

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### PLATE 16

- FIG. 1. Specimen of colon from a patient (A 38-328) who died from acute non-specific ulcerative colitis. The ulcers have been painted with India ink to make them apparent and show more clearly the three rows of lesions. These rows of ulcers lie directly over the teniae.
- FIG. 2. Section of colon from a patient (A 37-371) dying from non-specific ulcerative colitis. There are two rows of ulcerations in the longitudinal direction of the intestine, and they lie directly over the teniae. The third tenial band was cut away when sections were taken. Between the two teniae shown there are no ulcerations, only edema and hyperemia of the mucous membrane.
- FIG. 3. This section is from the region of a tenial band in a patient (A 37-108) who succumbed to chronic non-specific ulcerative colitis. The ulcerative process is shown directly over a tenia.



1



2



3

PLATE 17

- FIG. 4. Mucous membrane of normal human colon. There is only a narrow space between the glands and numerous goblet cells are present.  $\times 200$ .
- FIG. 5. Portion of mucous membrane from a case (A 38-10) of acute ulcerative colitis. Note the absence of goblet cells. This is similar to the changes produced by spasm alone in an explant of the dog's colon.  $\times 200$ .



4



5

Lium and Porter

Etiology of Ulcerative Colitis. III



## POLYARTERITIS NODOSA \*

### REPORT OF CASE

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A patient who had been under observation at the Massachusetts General Hospital for asthma for 8½ years finally died of polyarteritis nodosa. The autopsy findings are considered sufficiently interesting and unusual to warrant reporting the case.

### REPORT OF CASE

*Clinical History:* A 30 year old white stenographer entered the hospital for the fifth time with the complaints of fever, pain in the chest, abdomen and joints, and recurring asthmatic attacks. For the 8½ years previous to her final entry she had suffered from recurring attacks of typical asthma. Her four previous entries had all been for this reason, and on three occasions she had been in status asthmaticus. She had been treated by various means, including psychotherapy, without lasting results. There was nothing in the physical examination or laboratory findings inconsistent with the diagnosis of asthma. Her blood had shown an eosinophilia varying between 5 and 13 per cent.

One month before final entry she experienced some nausea and, for the first time, developed pain in the right lower chest and upper quadrant which radiated around to the back. During the next week the pain centered in the left lower chest posteriorly, and she began to have intermittent fever. She also had some stiffness in the shoulders. Three days before entry the elbows and wrists became red, swollen, stiff and painful. The following day painful red spots appeared beneath the nails of the fingers and toes. The evening before entry an asthmatic attack recurred in severe form and the elbows and wrists became very painful. She also had some nausea and vomiting, and had lost about 20 pounds in weight during the previous 4 months.

*Physical Examination:* This revealed a poorly developed and nourished woman in considerable respiratory distress. The lips and mucous membranes were pale and the nail beds were cyanotic. On the finger-tips and toes were many ham-colored lesions grouped together but not elevated. There were a few similar discrete lesions on the feet, and there were many petechiae beneath the fingernails and toenails and on the abdomen. A few, moist and sibilant râles were present in the lungs. No masses could be palpated in the abdomen, but there was diffuse tenderness and spasm in the epigastrium and right upper quadrant. The wrists were slightly stiff, tender, and painful with movement, and there was slight swelling on the dorsal aspects. Motion of the elbows was very painful so that they could not be fully extended. The blood

\* Received for publication August 3, 1938.



pressure was 95/60, the pulse 120, the temperature 102° F., and the respirations 28.

*Laboratory Examinations:* The urine had a specific gravity of 1.022, contained 1+ albumin, and showed occasional red cells, white cells and granular and hyaline casts. The blood showed a red cell count of 4,000,000 with 75 per cent hemoglobin and a white cell count of 43,000, with 60 per cent polymorphonuclears, 18 per cent lymphocytes, 2 per cent monocytes and 20 per cent eosinophils. The platelet count was slightly below normal. The guaiac test on the vomitus was negative and on the stool 1+ positive. A blood Hinton test was positive, and a Wassermann test negative. The non-protein nitrogen of the blood was 24 mg. per cent, the protein was 61 per cent, and the chlorides were equivalent to 103 cc. of N/10 sodium chloride. Several blood cultures were negative.

An electrocardiogram showed moderate to marked left axis deviation and was interpreted as consistent with coronary heart disease.

A roentgenogram of the chest showed marked increase in the size of the heart, particularly on the right side in the region of the pulmonary conus. The hilar shadows were slightly increased in width and there was marked increase in the lung markings, particularly in the hilar regions and lower lung fields. Diffuse haziness was present above the diaphragm suggestive of a pneumonic process. Three weeks later another roentgenogram showed disappearance of the apparent pneumonic process with persistence of the other findings. A postmortem roentgenogram showed the hilar shadows to be smaller and the lung markings to be essentially unchanged. In the peripheral lung fields there appeared to be an early miliary process.

*Course of Illness:* During her stay in the hospital the white count varied between 25,000 and 45,000, the eosinophils rising from the initial 20 per cent to 40 and 50 per cent. The pulse remained elevated although the temperature fell to normal on the 11th day. The abdominal pain continued and spread into both flanks. Both the liver and spleen became easily palpable. She had transient blindness of the left eye lasting for 2 days. On the 36th hospital day she developed severe, migrating neuritic pains in the extremities which caused a great deal of distress. The course of illness was marked by progressive weakness and emaciation, and death occurred on the 45th hospital day. A clinical diagnosis of polyarteritis nodosa was made on the basis of the history of severe asthma, irregularly distributed pain in the joints and muscles, pain in the abdomen and chest, skin lesions, evidence of renal and cardiac damage, emaciation, and marked eosinophilia.

## POSTMORTEM EXAMINATION

At autopsy no gross arterial lesions were discovered. The terminal ileum and colon showed numerous superficial areas of ulceration which were for the most part oval in shape, measuring up to 1.5 cm. in length, with long axes running transversely.

The heart weighed 325 gm. The right ventricle was somewhat dilated, and embedded beneath the endocardium of the left ventricle was a firm, dull gray nodule measuring 2 mm. in diameter.

Both lungs were similar in appearance. Scattered throughout the parenchyma, most marked in the right lung and least marked in the left upper lobe, were numerous grayish white nodules measuring 0.2 to 1 mm. in diameter, some of which were confluent. In the inferior edge of the left lower lobe was a triangular area of obvious infarction measuring 1.5 by 1 by 0.5 cm. No emboli were found.

In the left lobe of the liver and adjacent portion of the right lobe were large reddish areas of infarction.

The spleen weighed 825 gm. It was very soft and friable and the normal architecture was completely obliterated by almost confluent round areas of soft white tissue measuring up to 3 mm. in diameter with intervening areas of dark red hemorrhagic tissue. The splenic vein and artery were negative.

The adrenals and pancreas were grossly negative, but the kidneys showed a number of small areas of infarction measuring up to 3 mm. in diameter.

Several peripheral nerves and specimens of muscle appeared negative in gross.

The brain showed an area measuring 1.5 cm. in diameter of recently clotted blood in the posterior portion of the left lenticular nucleus.

### MICROSCOPIC EXAMINATION

Microscopic lesions characteristic of polyarteritis nodosa were found in the heart, lungs, liver, pancreas, adrenals, kidneys, muscles, peripheral nerves and brain, but were not found in the ovary, aorta, lymph nodes, intestine or bone marrow. The size of the arteries affected varied considerably but none of them was larger than 1.5 mm. in diameter and few, if any, arterioles were involved. None of the affected vessels could be identified as veins. Although the colon showed extensive ulceration similar to that seen in tuberculosis, no microscopic arterial lesions could be demonstrated. Only ulceration and inflammation of an entirely non-specific nature was seen. No gross or microscopic lesions were demonstrated in large vessels such as the coronary, mesenteric or renal arteries.

In this study four definite stages of the disease were found: (1) early acute stage; (2) advanced acute stage; (3) granulating stage; and (4) healed stage.

What appears to be the very earliest acute vascular lesion was limited to the vessels supplying the skeletal muscles. It consisted of apparent disintegration of the intima and innermost layers of the media of the arteries with infiltration of these areas by polymorphonuclear leukocytes, eosinophils and fibrin. In some blood vessels the internal elastic lamina remained intact, although it was usually partially destroyed. The middle and outer layers of the media were unaffected, and there was no periarterial thickening or inflammation. Many of the vessels were thrombosed.

Advanced acute lesions also occurred in the muscles, and in the pancreas, brain and nerve bundles. At this stage the intima and inner layers of the media were affected in all cases, and there were varying degrees of disintegration of the internal elastic lamina and muscular coats with loss of architecture and disappearance of many of the smooth muscle cells. The wall and often the adventitia were infiltrated by eosinophils and polymorphonuclear leukocytes. The mural disintegration did not always involve the entire circumference of the vessel. Frequently one-third or more of the circumference appeared perfectly normal with the muscle fibers and elastica preserved in their orderly arrangement. Often apparent aneurysmal dilatation of the affected segments of the vessels was seen, but there was no interstitial hemorrhage, probably because almost all these vessels were thrombosed. The lesions were characteristically segmental in their distribution. Longitudinal sections of the arteries showed involvement of as much as a millimeter of the wall without any damage to the adjoining segments on either side of the lesion.

Granulating lesions were seen in the lungs, adrenals and kidneys. There was ingrowth of fibroblasts into the affected portions of the wall and adventitia with decrease in the numbers of eosinophils and polymorphonuclear leukocytes, and increase in lymphocytes, plasma cells and large mononuclear cells. In this stage the adventitia was almost invariably involved in the process. In the more advanced granulating lesions the necrotic tissue of the acute stage was entirely replaced by cellular fibrous tissue which extended into the adventitia. The areas of the wall unaffected in the acute stage remained normal in appearance. There was also marked thickening of the intima by moderately cellular fibrous tissue, often with complete occlusion. It is difficult to say whether

the appearance was due to organization of a preexisting thrombus with or without recanalization or to intimal proliferation in a vessel that was not thrombosed. In at least a few of the more acute lesions the occluding acute thrombi showed very definite beginning organization, indicating that this process was responsible for some of the luminal occlusion seen in the later stages. An artery in the adrenal showed infiltration of the entire media with densely packed, blue staining, fibrin-like material. The rest of the lesion showed an advanced stage of granulation.

Healed lesions were seen in the liver and heart. In the liver the granulation tissue was replaced by dense, relatively acellular fibrous tissue containing a few pigment-bearing mononuclear cells and occasional foreign body type giant cells. In some of the lesions part of the media of the blood vessels could be identified with the underlying elastica intact. The lumen was almost invariably occluded and recanalized. In the heart all the lesions were healed, and only a few of the smallest arteries were affected. These, however, showed no definite involvement of the muscular layer. Marked fibrous thickening of the intima with partial or complete occlusion, or occlusion followed by recanalization was present. In some, the internal elastic lamina was partially destroyed but in the majority it was intact.

The histological appearance of the spleen was most unusual. With the exception of a few very small follicles around persisting trabeculae, the normal architecture was almost completely obliterated. There was massive infiltration of the parenchyma by large numbers of monocytes and red cells with a few lymphocytes and moderate amounts of fibrin. In some areas the fibrin was arranged concentrically around collections of cells giving a follicle-like appearance. There were no areas of necrosis, no fibrosis, and no suggestion of tubercle formation. Sections stained for tubercle bacilli showed no acid-fast organisms, and material inoculated into guinea pigs failed to produce tuberculosis. Very extensive degeneration of the walls of many of the arterioles was present, but no infiltration by eosinophils or polymorphonuclear leukocytes and no periarteriolar thickening was seen. Many arterioles were almost unrecognizable. Some of them were thrombosed and only a few were normal in appearance. However, no definite specific lesions such as occurred in other organs could be identified. Some

of the small arteries showed infiltration of the adventitia by lymphocytes and plasma cells, and in many of the veins there were small subendothelial collections of mononuclear cells elevating the endothelium and encroaching upon the lumen.

In the lungs were widespread miliary foci of organizing pneumonia involving areas which would normally be occupied by one to ten alveoli, although some of them were considerably larger. These foci consisted of interlacing fibrous tissue with a few mononuclear cells and eosinophils, occasional small foreign body type giant cells, and small amounts of fibrin. No areas of caseation necrosis were present, and no acid-fast organisms could be identified in sections stained for tubercle bacilli. In many of the larger branches of the bronchial arteries measuring 0.5 to 1.5 mm. in diameter were specific lesions in the advanced stage of granulation, most of which were located near the hili. In some of the pulmonary arteries were lesions that appeared to be organized mural thrombi. They were suggestive of specific lesions, but without destruction of the arterial walls or infiltration by inflammatory cells they could not definitely be considered as such.

Varying stages of ischemic necrosis were present in the heart, liver, kidneys, adrenals and pancreas. However, the stages of infarction did not necessarily correspond with the apparent age of the corresponding arterial lesions. In the heart where the arterial lesions were healed were a few areas of acute necrosis of the muscle bundles, although there were present also numerous small areas of healed infarction. In the liver all the infarction was early; whereas the majority of the arterial lesions were healed. In the kidneys areas of early infarction and also areas where the tubules were degenerated without damage to the glomeruli or interstitial tissue were present. The adrenals showed several areas in the cortex where the cortical cells were completely replaced by macrophages filled with fat. A few small areas of early ischemic necrosis were seen in the pancreas.

#### COMMENT

In all the arterial lesions observed there was some degree of damage, with or without repair, of the intima and almost invariably of the inner layers of the media. Aside from the evidence already offered this would indicate that in this case the primary lesion

occurred in the intima and progressed outward through the wall to the adventitia.

Arkin<sup>1</sup> has classified the lesions in four stages. The first is an alterative degenerative stage. The elastica interna and innermost layers of the media become edematous and infiltrated by a thready fibrinous exudate, and part of the muscle layer of the entire wall undergoes a coagulation necrosis with beginning infiltration by leukocytes. This stage corresponds roughly with the early acute stage described in this case but differs from it in that the lesion is not confined to the innermost layers of the artery. Arkin's acute inflammatory, granulation and healed stages correspond to the three later stages described in this case.

In any inflammatory process progression from the acute to the healed stage occurs, and the lesion of polyarteritis nodosa is no exception to this. The effect of the toxic agent is limited by the inflammatory response, and greater or less damage is done depending on the various factors operative in each individual instance. The extent of the healed lesion depends directly on the amount of damage done in the acute stage.

An unusual feature of this case was the coincident existence of all stages of the arterial lesions. In many of the cases reported<sup>2-8</sup> with detailed descriptions of the histological findings, the lesions showed some variation in their stage of development, but in this case variation from the earliest acute to the healed stage was observed. However, with one exception this marked variation did not occur in the individual organs. Except in the liver, all the arterial lesions of each individual organ were roughly in the same stage. Only acute lesions were seen in the pancreas, brain, muscles and nerve bundles; only granulating lesions were seen in the lungs, adrenals and kidneys; and only healed lesions were seen in the heart. The majority of the lesions in the liver were healed, but a few were acute. This restriction of lesions to one stage in the individual organs cannot be readily explained. From a purely speculative point of view it may possibly have some bearing on the pathogenesis of the disease. Although the lesions are widely distributed throughout the body with the primary damage to the arteries, each individual organ as a whole may have a specific reactivity or resistance which determines its response to the toxic agent.

The lungs in our case showed an unusual X-ray appearance similar to findings in the case reported by von Conta<sup>9</sup> (quoted by Spiegel<sup>10</sup>), and in the case reported by Herrman<sup>11</sup> and Wiener.<sup>4</sup> Von Conta described shadows ranging in size from "a lentil to a cherry" located near the hilus with specific lesions of the pulmonary arteries at autopsy. Herrman and Wiener described a diffuse perivascular infiltration spreading out uniformly and equally from both hilar regions with specific lesions of the bronchial and pulmonary arteries. In this case the hilar shadows were slightly increased in width with marked increase in the lung markings, particularly in the hilar regions and lower lung fields. A postmortem film showed a generalized early miliary process. At autopsy there were numerous specific lesions of the bronchial arteries near the hili with miliary foci of organizing pneumonia throughout the parenchyma. The pulmonary arteries and peripheral branches of the bronchial arteries showed very little involvement. This appearance by roentgen examination of perivascular infiltration spreading out from the hilar regions may be helpful in establishing the diagnosis in a suspected case of polyarteritis nodosa. It cannot, of course, be considered diagnostic.

The gross appearance of the spleen was the most striking feature of the case at autopsy. It was enlarged to more than four times its normal size and showed lesions suggestive of very extensive tuberculosis of an unusual variety. Microscopically the entire organ was involved by a hemorrhagic, apparently inflammatory process, which had no resemblance to tuberculosis or any other specific disease.

The gross autopsy findings as a whole simulated tuberculosis more closely than any other condition. The miliary process in the lungs, the splenic lesions, the small infarcts in the kidneys, and the ulceration of the colon gave a combined picture which could well have been produced by widespread tuberculosis. The infarcts of the liver, the cerebral hemorrhage and the absence of any primary focus of tuberculous infection were, of course, incompatible with the diagnosis.

A note may be appended about nomenclature. Many of the older writers, including Lamb,<sup>12</sup> believed the lesion to be primarily periarterial. More recent evidence presented by numerous writers and the findings embodied in this report make it reasonably cer-

tain that the lesions are not primarily periarterial. In 1907 Dickson<sup>13</sup> suggested that the name polyarteritis nodosa be adopted. Periarteritis nodosa is, without much question, a misnomer and for that reason should be dropped. Polyarteritis nodosa would be a suitable substitute.

### SUMMARY

A case of polyarteritis nodosa is reported with clinical findings which made possible an accurate clinical diagnosis. The outstanding symptoms and signs were a history of severe asthma, irregularly distributed pain in the joints, muscles, abdomen and chest, skin lesions, evidence of renal and cardiac damage, emaciation and marked eosinophilia.

A detailed description of the gross and microscopic autopsy findings is given with comments on a number of unusual features of the case. These unusual features are: (1) Evidence that the primary lesion occurred in the intima and inner layers of the media and progressed outward through the wall to the adventitia; (2) occurrence of all stages of arteritis from the earliest acute to the healed, but restriction of the lesions in each individual organ to roughly one stage; (3) remarkable parenchymatous lesions of the lungs and spleen; and (4) resemblance of the gross autopsy findings to widespread tuberculosis.

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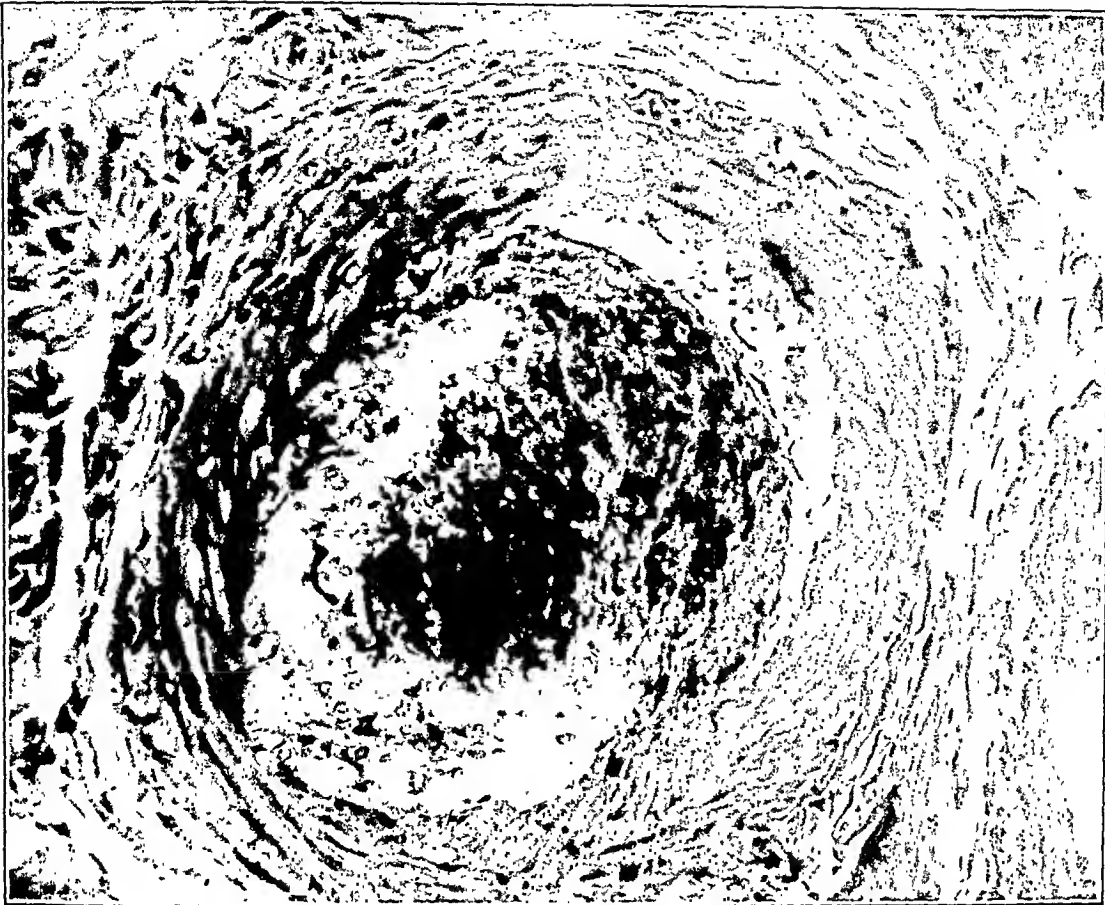
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## DESCRIPTION OF PLATES

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### PLATE 18

- FIG. 1. An early acute lesion in a small artery of the biceps muscle. The intima and inner layers of the media are disintegrated and the lumen is occupied by a recent thrombus. There is no degeneration or cellular infiltration of the outer layers of the media, and no periarterial inflammation. Phloxine-methylene blue stain.
- FIG. 2. A somewhat more advanced acute lesion of an artery 1 mm. in diameter in a nerve bundle. In the upper part the destruction of the wall is almost complete and there is beginning periarterial inflammation. Phloxine-methylene blue stain.



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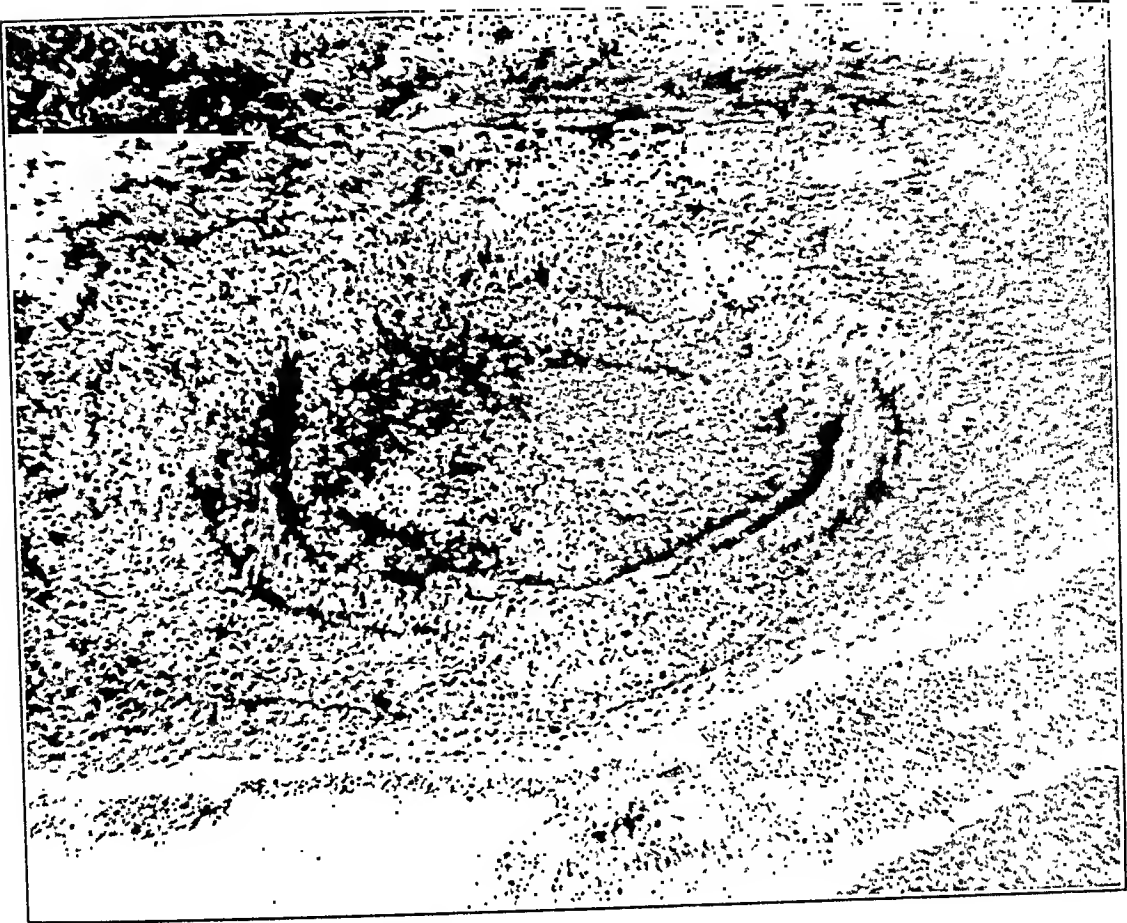


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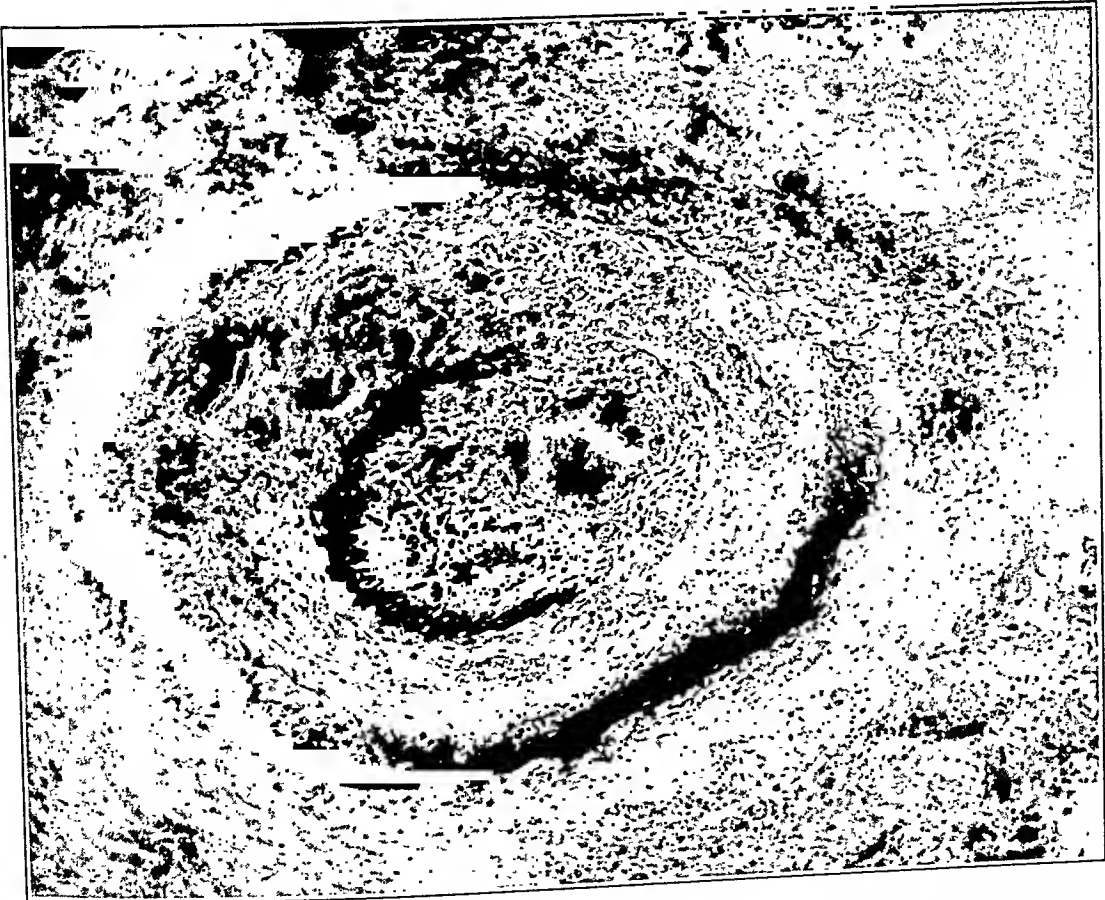
PLATE 19

FIG. 3. A granulating lesion of an artery 1 mm. in diameter in the kidney. There is almost complete destruction of the media except for a small area on the right where a segment of persistent media can be made out between the elastic lamellae. Verhoeff's elastic tissue stain.

FIG. 4. An advanced granulating lesion of a bronchial artery 1 mm. in diameter. Most of the inflammatory cells have disappeared and many small vessels have developed, partly in the granulation tissue which has replaced the media, and partly as recanalizing vessels in the thrombus. Verhoeff's elastic tissue stain.



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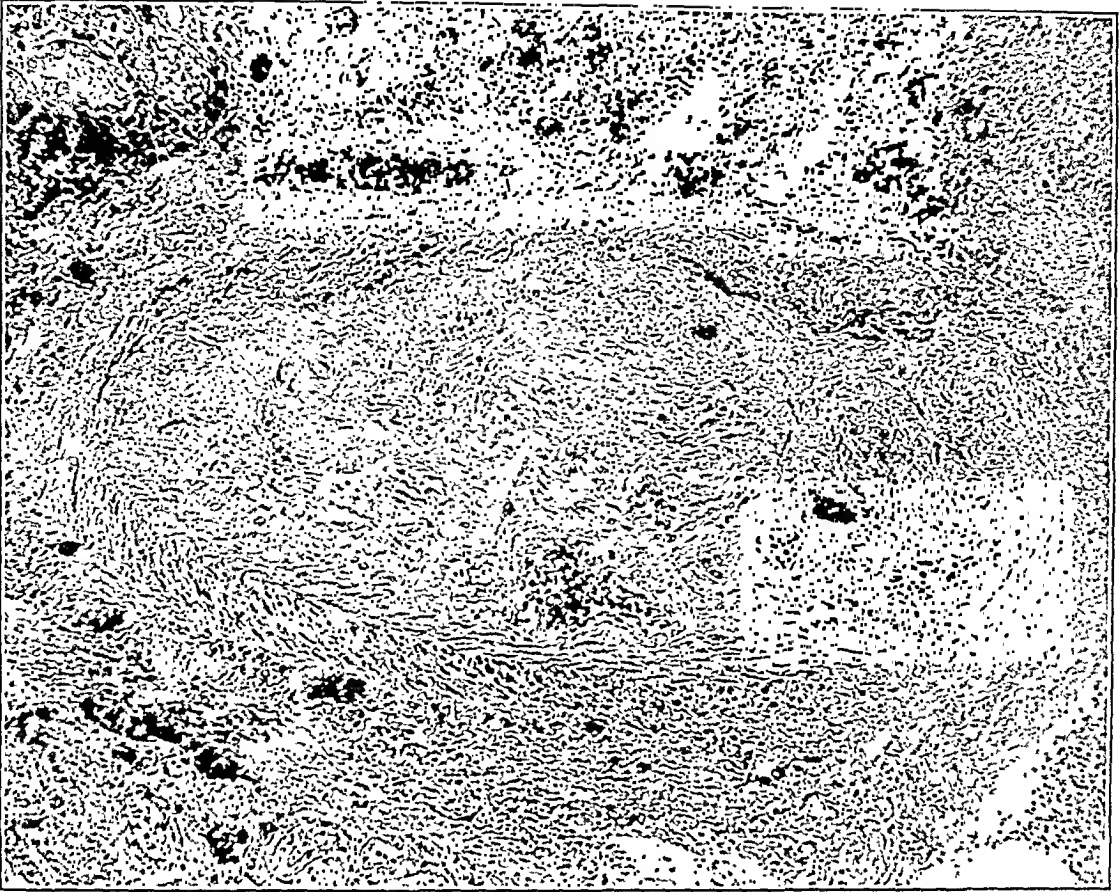
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Polyarteritis Nodosa

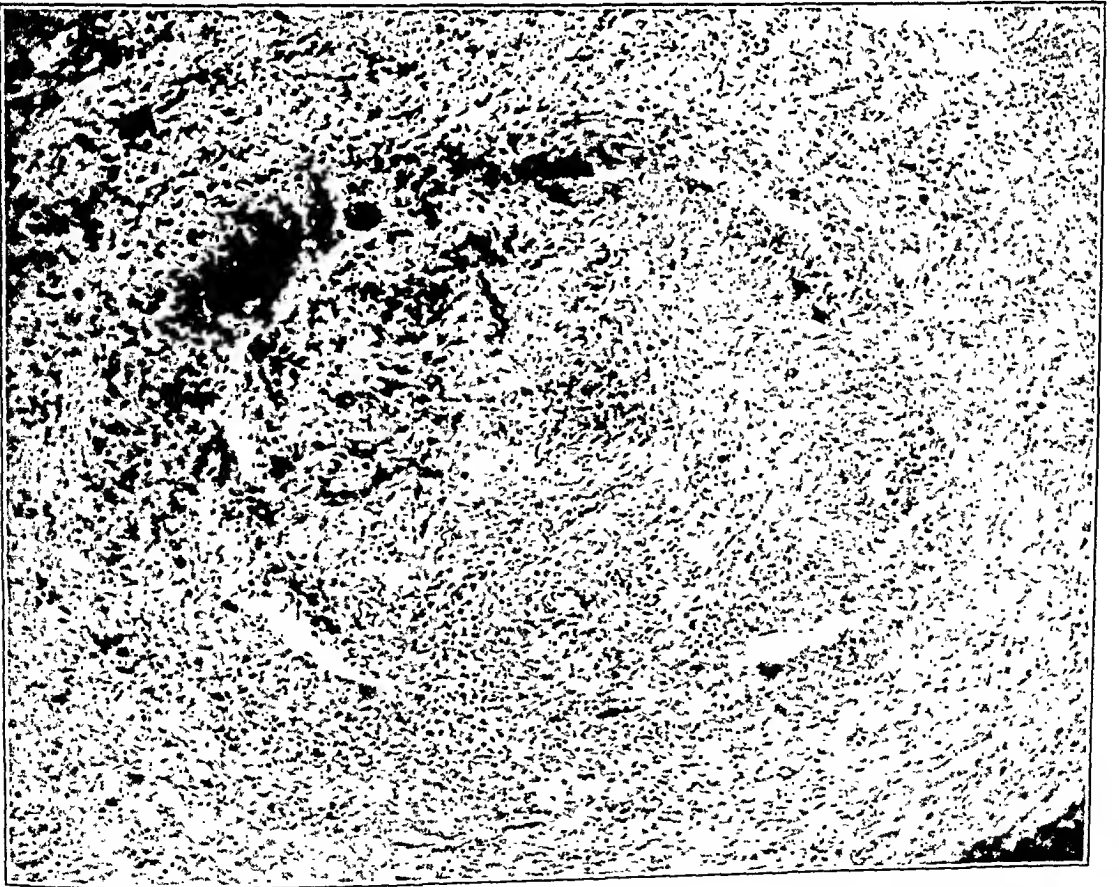
PLATE 20

FIG. 5. An advanced granulating lesion of an artery 1 mm. in diameter in the kidney. Most but not quite all the inflammatory cells have disappeared. Some of the smooth muscle of the media is preserved. Phosphotungstic acid hematoxylin stain.

FIG. 6. An almost healed lesion of an artery 1.5 mm. in diameter in the liver. Several foreign body giant cells are seen in the wall. Hematoxylin-eosin stain.



5



6



# METASTASIZING FIBROLEIOMYOMA OF THE UTERUS\*

## REPORT OF A CASE AND REVIEW OF THE LITERATURE

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Dissociation between clinical and histological malignancy in neoplasms reaches its acme in the "benign metastasizing tumors" in which the histological appearance is benign but which metastasize and prove to be clinically malignant. Among these tumors are certain chondromas, some angiomas, and "metastasizing adenomas" of the thyroid. To these must be added a few reported cases in which fibroleiomyomas of the uterus and myomas of other locations have metastasized. This report deals particularly with the problem of metastasizing fibroleiomyomas of the uterus.

As used in this paper, the term "metastasizing fibroleiomyoma" of the uterus refers to a tumor composed histologically, in both the primary growth and its metastases, of benign appearing, fully differentiated smooth muscle cells and dense connective tissue. This distinguishes it from primary leiomyosarcoma of the uterus and from sarcomas arising in fibromyomas or so-called sarcomatous degeneration of fibroids, in both of which the tumor cells have histological characteristics of malignancy in the primary tumor as well as in the metastases. Such unquestionable sarcomas lie beyond the scope of this study and will not be stressed. Instances are also recorded in the literature in which a uterine tumor was removed and called a fibromyoma after histological study, but in which local recurrences or metastases later showed histological evidence of anaplasia and malignancy. With such cases also we are not concerned in this report.

Metastasizing fibroleiomyomas of the uterus have been seen and described by several observers but their occurrence is so rare that some oncological authorities have never seen a case and have expressed doubts as to their existence. Thus Ewing<sup>1</sup> in discussing the possibility that uterine leiomyomas of benign appearance might metastasize states: "So far as I have been able to learn no

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case has been fully studied in which definite variations from the usual structure of leiomyoma were wanting, although in several instances these variations have not been very pronounced." Stout, in discussing the same problem, writes <sup>2</sup>: "While Jacquin has been able to collect 5 cases of tumors which morphologically were fibromyomas and yet metastasized and recurred always as unaltered myomas, such reports must be exceptionally rare and they leave one with the suspicion that there may be some change which has been overlooked even though the cases were reported by such keen observers as Langerhans, Minkowsky, Schlagenhauser and von Franque." Meyer <sup>3</sup> and Albrecht <sup>4</sup> also believe that metastasis of mature myoma cells has not been proved up to the present. They believe that multiple sections from the primary tumors in the uterus in suspected cases would reveal a sarcoma in some portion. They fail, by this explanation, to account for the benign appearance of the structure in the metastases in these cases, and their lengthy discussions together with that of Raab <sup>5</sup> are beside the point.

Against these opinions stand the written words of a host of authorities who accept these tumors. Among them are Kaufmann, <sup>6</sup> Borst, <sup>7</sup> Aschoff, <sup>8</sup> Wolff, <sup>9</sup> Ribbert, <sup>10</sup> and others.

The question can then legitimately be asked whether or not such a tumor exists in the uterus or elsewhere. Since appeal to authority cannot settle this question, a critical evaluation of the cases reported in the literature, together with the new evidence obtained by study of a case, may, we hope, help clarify the subject. My attention was drawn to this problem after studying a case which seemed to fulfill the criteria for this type of neoplasm. The microscopic sections have been seen by about twenty pathologists who, suspecting that a benign myoma would not be submitted for pathological opinion, were nevertheless unable to make a diagnosis of malignancy.

Because of their rarity metastasizing fibroleiomyomas of the uterus are not of great practical importance. To the oncologist, however, they are of great interest because they illustrate certain limitations in the purely histological diagnosis of malignant disease, emphasize the inadequacy of the classical criteria for malignancy, as opposed to benignancy, remind him to be particularly cautious in the diagnosis of certain types of tumors, and

illustrate differences from accepted biological behavior in a number of interesting respects.

This paper deals with the case of a patient who exhibited the clinical picture of chronic pulmonary obstruction with cor pulmonale and polycythemia, and who died with right sided heart failure. The changes in the lungs during life suggested most strongly a chronic pulmonary infection. A symptomless pelvic mass was recognized during life but its relation to the changes in the chest was uncertain prior to autopsy. From a uterus the site of multiple tumors resembling fibroleiomyomas, massive metastases occurred to the lungs and to a tracheobronchial lymph node. The microscopic appearance of the metastases and of the uterine tumors was that commonly seen in uterine fibroids, the cells being benign in appearance and fully differentiated.

### REPORT OF CASE

*Clinical History:* M. B., a housewife, aged 36 years and of English descent, was under observation at the University of Chicago Clinics (Unit #100,419) from March 12, 1934, until her death 15 days later. She gave a history of dyspnea, wheezing respirations, nocturnal headaches and nosebleeds during the preceding 18 months, a productive cough for about a year, marked orthopnea for 9 months and swelling of the ankles and abdomen for 6 months.

The patient had a prolonged chill accompanied by transient pain in the chest in May, 1933. During that summer she had a period of afternoon rise in temperature. She lost 77 pounds in weight between February and October, but had regained 25 pounds before her present hospital admission 5 months later. Her sputum was abundant, thick and yellowish, but not bloody. After study of X-ray films a physician had made a diagnosis of tuberculosis more than a year before her death, but no tubercle bacilli and no fungi could be found in the sputum. This diagnosis was abandoned by him in October, 1933, when X-ray films showed a slight recession instead of the expected advancement in the lung lesions. X-ray therapy to the chest was then followed by temporary subjective improvement. In January, 1934, the heart was found to be enlarged and she was decompensated, having dependent edema and an enlarged liver.

The patient stated that she had had mumps, chickenpox, measles and meningitis in childhood. She had never been pregnant. Prior to January, 1933, her menstrual periods had been regular at 30 day intervals and of 5 days duration. In January, 1933, she had a menstrual period accompanied by nosebleeds. The next periods were in May, June and July, 1933. There had been none since. Her husband was in good health. Her mother had died of diabetes at the age of 57 years.

On physical examination the patient appeared acutely ill with cardiac decompensation and advanced pulmonary disease. She was cyanotic, very dyspneic and preferred the sitting position. Examination of the eyegrounds showed tortuous vessels and an aneurysm of the left superior papillary artery.

The fingers were clubbed. Expansion of the chest, especially on the left, was diminished. Inspiration was short and expiration was prolonged. There was dullness to percussion with increased tactile fremitus over the base of the lungs. Coarse and fine moist râles, not removed by coughing, were heard in these regions. The border of the left side of the heart was 2 cm. beyond the mid-clavicular line. No murmurs were heard. The rhythm was normal but the rate was 110. The blood pressure was 130/80. An electrocardiogram showed a right axis deviation with inversion of T in leads II and III. The abdomen was greatly distended and there was some question as to whether it contained free or encysted fluid. The liver extended 5 cm. below the costal margin. The lower extremities showed only a slight edema. A hard tumor mass was palpable above the pubis to the left of the midline. On vaginal examination the cervix was found to be pulled up and a hard mass replaced the uterus.

The erythrocyte count was 5,680,000 and the hemoglobin was 101 per cent. The red cells showed a slight microcytosis and polychromatophilia. The leukocyte count varied between 6100 and 9200 with a differential count of 74 per cent neutrophils, 18 per cent lymphocytes, 6 per cent monocytes and 2 per cent basophils. The urine, except for a few white cells, was negative. No acid-fast bacilli or fungi were found in the thick sputum on repeated examination. The blood Wassermann and Kahn tests were negative. On attempted abdominal paracentesis a resistant mass was encountered and only a little bloody fluid which contained no unusual cells was obtained.

X-ray films made after admission showed many areas of increased density scattered throughout both lungs (Fig. 1). They were more marked in the lower lobes. There was a suggestion of bilateral cavitation. It was impossible to differentiate between tuberculosis and neoplasm from the films, although the picture was unlike that of the usual metastases to lungs. X-rays made by another laboratory in February, 1933 revealed an involvement of the lungs which already at that time resembled and was almost as spectacular as the condition shown on the last films.

The patient became more cyanotic and dyspneic, symptoms which were alleviated only by the use of the oxygen tent. The temperature varied between 100 and 102° F. and death occurred 15 days after admission to the hospital.

### POSTMORTEM EXAMINATION

The body was obese, weighing 187 pounds and measuring 162.5 cm. in length. The abdomen was protuberant with bulging in the flanks and a palpable mass was present in the midline extending upward to slightly above the level of the umbilicus. The legs and posterior parts of the body were edematous. The head and neck, the lower extremities and the back showed a marked cyanosis.

The pleural cavities had a few fibrous adhesions. The lungs were heavy and voluminous. They were everywhere studded with firm, pink to reddish tumor nodules varying in size from just visible to larger masses measuring up to 5 cm. in diameter. Intermingled with these solid masses were thin walled, semitranslucent

emphysematous sacs which showed the same variation in size. They collapsed on slight pressure. On forcing air into the lungs through the trachea these air cysts were reinflated, demonstrating their communication with the bronchial tree. They again collapsed when the pressure was released. Tumor nodules of both types, solid and cystic, projected above the surface of both lungs. A few tumors of each type were pedunculated, particularly along the lower lung margins (see Figs. 2, 3 and 4).

On the cut surfaces the solid tumors were found to be scattered throughout all lobes, but large air cysts were found only near the surface of the lung. The solid tumors were sharply demarcated but had no visible capsules. They were a reddish gray in color and the cut surface showed irregular whorls resembling a fibromyoma. Between these solid tumor nodules the parenchyma of the lung presented a honey-comb appearance because of small, thin walled air sacs. Most of the surface made by cutting was occupied by either solid tumor or cystic spaces so that there were only small, irregularly interspersed areas which appeared to be composed of functioning lung parenchyma. Frothy bloody fluid escaped from the surfaces. Each lung presented the same appearance. The right lung in addition contained a large firm mass measuring 13 by 12 by 12 cm. in the lower and lateral part of the upper lobe. This tumor contained a large cavity that was filled with a thick mucinous fluid which was sterile on bacterial culture. The walls of this cavity, while generally up to 2 cm. in thickness, showed portions as thin as 3 mm. Adjacent to this large tumor were several smaller tumors with mucinous material in their centers.

The tracheobronchial lymph nodes were slightly enlarged and several nodes showed areas of tumor tissue under the capsule resembling that seen in the lung.

The emptied heart weighed 475 gm. The increase in weight was due mainly to hypertrophy of the right ventricle, which, although the chamber was dilated, measured from 8 to 9 mm. in thickness. The left ventricle was normal in size and its wall measured 14 mm. The heart valves appeared normal. The myocardium showed no scarring and microscopically only a few small foci of mononuclear cell infiltration and a few small focal areas of fatty change were seen. The coronary arteries and aorta showed a

minimal amount of atheromatosis. The pulmonary artery and its main branches appeared normal. Their walls were thin and elastic.

The abdominal cavity contained about 600 cc. of a slightly turbid, yellowish brown fluid. The omentum was free. The intestines were cyanotic and were displaced upward by a mass arising in the pelvis from the uterus.

The liver weighed 1810 gm. Microscopically a marked hyperemia in the centers of the lobules with some necrosis in these areas, slight fibrosis, and marked fatty changes were present. The gall bladder and extrahepatic bile ducts appeared normal.

The spleen weighed 340 gm. It was cyanotic and firm and microscopically showed a diffuse fibrosis. Accessory spleens 1 and 2 cm. in diameter showed chronic passive congestion similar to that observed in the main spleen.

The gastric mucosa showed a diffuse hyperemia and numerous petechial hemorrhages. The duodenal mucosa was intensely hemorrhagic and exhibited a number of irregular erosions measuring up to 2 cm. in diameter. Throughout the remainder of the gastro-intestinal tract the walls showed variable amounts of edema, hyperemia and petechial mucosal hemorrhages.

The kidneys were cyanotic and showed also cloudy swelling with a moderate amount of granular degeneration of the epithelium of the convoluted tubules. The lower urinary tract appeared normal.

The uterus was enlarged, measuring 18 by 16 by 10 cm. (Fig. 5). Its upper surface was covered by smooth peritoneum. The uterine mass was nodular but fairly symmetrical except for nodules that projected into the right broad ligament. The surfaces made by cutting showed that it was composed of many firm nodules from a few mm. to 5 cm. in size, embedded in a fleshy fibrous stroma. The tumor nodules shelled out easily. Many of them were globular while others were elongated and irregular in contour. Closer examination showed that the tumor nodules occupied the anterior, lateral and upper walls of the uterus, and that the posterior myometrium was normally thin and muscular, measuring just over 1 cm. in thickness. The tumor nodules resembled fibroids, being firm, reddish gray, and composed of interlacing whorls of tissue. One tumor nodule showed an area of liquefaction necrosis in its center, and several others, including those

which projected into the broad ligament, were edematous. The veins in the right broad ligament were dilated and contained red ante mortem thrombi.

The uterine cavity was elongated and distorted. The endometrium was intact and normally thin, and except for a slight hyperemia was not unusual. The fallopian tubes were bilaterally thin, slender and of uniform caliber; their fimbriated ends were open and free. The ovaries were of normal appearance and on microscopic examination appeared to be functioning, having numerous ova and several small follicular cysts.

The thymus, adrenals, mammary glands, pancreas and pituitary appeared normal. The thyroid microscopically showed a diffuse and focal dense connective tissue increase with hyalinization in some places and lymphocytic foci in others. The brain showed only an anomaly in the circle of Willis. Instead of a single anterior communicating artery joining the anterior cerebral arteries there was a network of arteries from which passed a single right anterior cerebral and two left anterior cerebral arteries, the latter communicating 1 cm. from their origin but still remaining separate trunks. The lymph nodes showed no changes except those mentioned with respect to the tracheobronchial nodes. The muscular system and skeleton likewise showed no abnormality. The bone marrow from a rib and from a lumbar vertebral body showed a hyperplasia of hemopoietic elements.

*Anatomical Diagnoses:* Multiple fibroleiomyomas of the uterus; multiple solid and cystic fibroleiomyomatous metastases to each lung and to the tracheobronchial lymph nodes; pulmonary emphysema and slight hyperemia; hypertrophy and dilatation of the heart, particularly of the right ventricle; generalized passive congestion; ascites; edema of the dependent parts of the body; parenchymatous degeneration of the liver and kidneys; petechial hemorrhages in the mucosa of the stomach, jejunum, ileum and colon; acute erosions of the duodenal mucosa; fibrous pleuritis; accessory spleens (2); and anomaly of the circle of Willis.

#### ESSENTIAL MICROSCOPIC FEATURES

Except for the tumors in the uterus, lungs and tracheobronchial lymph nodes, the microscopic changes have been briefly given and will not be repeated. Tissues were fixed in Zenker's and

Bouin's solutions and in formalin. Embedding was in paraffin and celloidin. Sections were stained by hematoxylin and eosin, phosphotungstic acid hematoxylin, Mallory's aniline blue stain for connective tissue, Van Gieson's connective tissue stain and Masson's trichrome method. The distribution and fate of the elastic tissue in the lung was studied by Weigert's method. Frozen sections of heart, liver and kidney were stained for fat. The autopsy was begun 3 hours and 20 minutes after death but all tissues were not fixed immediately so that the results of the differential staining were not perfect.

*Uterus:* Sections from sixteen locations in the uterine tumors show, except for minor differences, the same microscopic picture. The tumors resemble the common fibroleiomyoma with the smooth muscle component conspicuous (Fig. 9). In some areas the tumor is very vascular and in these places the cells tend to be shorter than elsewhere and to exhibit a tendency to a perivascular arrangement. Dense connective tissue stroma with much collagen is abundant in some nodules, while others show much edema fluid separating the tumor cells. Other degenerative changes, common in fibroids, except for the liquefaction necrosis seen in one tumor nodule, are absent. In one section made from the tumor which extended into the broad ligament superficial infiltration of tumor cells into skeletal muscle is noted although this had not been observed on gross dissection. Invasion of tumor cells through the wall of a uterine vein to form a sessile mass projecting into the circulating blood is seen in one section.

The predominant cytological constituent of the tumor is smooth muscle. Anaplasia is absent in the tumor cells. The nuclei are regular, symmetrical and cigar-shaped, and lie parallel. There are more nuclei per unit volume than are usually seen in proliferating tissues composed of young connective tissue, and the nuclei contain more chromatin than do young fibroblasts. Mitotic figures are rarely found. The cells form fascicles which interweave. Among the muscle cells are connective tissue cells, which are numerous in some areas. Mast cells are also scattered throughout the sections.

The stains for fibrillar cytoplasmic material show that there are myofibrils within tumor cells. They can be seen where the cells are cut transversely and they also appear where the cells are

cut longitudinally, although here their localization within the cytoplasm of the cells is less certain. The myofibrils, in many places, appear to be degenerating, being clumped near the nucleus and taking a reddish brown instead of a blue color with the phosphotungstic acid hematoxylin stain. Coarser myoglia fibrils, which stain blue to black with the same stain, are visible on the surfaces of the cells in many regions.

The chromatic properties of the tumor cells in the various stains, with respect to both the nucleus and the cytoplasm, are best compared with the blood vessel walls in the same sections. They are seen to resemble the smooth muscle cells of the tunica media and not the young fibroblasts of the tunica adventitia. The fibrils of the tumor cells have the tinctorial qualities of the elastic fibers of the blood vessel wall, where such are visible. Dense connective tissue with collagen is present in all sections. In some regions it is abundant while in the younger, perivascular regions very little is seen. The relationship and relative amounts of the various types of fibrils are easily seen in edematous regions where the cells have been teased far apart and conform essentially to the above descriptions.

Sections through the endometrium show an infiltration by plasma cells, lymphocytes and neutrophils, numerous thin walled blood vessels and an atrophic epithelium. The cervical glands are also atrophic. Numerous sections through the para-uterine vessels reveal many veins and one artery to be thrombosed by recent or by organizing thrombi not containing tumor at the levels studied.

*Lung:* Except for less vascularity and an even more highly differentiated cell morphology, the cytological appearance of the lung metastases resembles that seen in the uterine tumors. The essential tumor cells appear to be smooth muscle with a stroma of varying amounts of dense connective tissue. These cells form circumscribed but encapsulated infiltrative nodules, mainly in the interstitial regions, but also in the region of perivascular and peribronchial lymphatic channels (Figs. 6 and 7). Some nodules are composed of solid masses of tumor cells, but in the majority are also found irregular spaces lined by flattened or cuboidal epithelium resembling that seen in chronically scarred lung tissue. These spaces resemble persistent alveoli which have become lined



by cuboidal epithelium. Most of these alveoli are collapsed, with opposing epitheliums in contact, but others are open and appear to contain either air or mucus. The nature of the remarkable air cysts described in gross is revealed by studying the forms these alveoli assume. The cystic tumors appear to represent enormously enlarged alveolar sacs. Their walls are composed of smooth muscle tumor cells which are greatly elongated and they are lined by flattened epithelial cells. Solid and cystic lung tumors, therefore, differ only in whether an enlarging alveolus persisted within the tumor nodule. The large mucus-filled tumor mass found in the right lung has lining cells which appear to have formed the mucus. The presence of myofibrils and myoglia fibers can be demonstrated in the lung metastases (Fig. 8). No tumor growth is noted within large blood vessels in the lung.

*Lymph Nodes:* Fibroleiomyomatous tissue is seen in the peripheral sinus and in an afferent lymphatic of a lymph node removed from the region of the bifurcation of the trachea (Fig. 10). The cells here also appear fully differentiated and benign. The specific fibrils for smooth muscle are present. Fibrous stroma is present and the tumor tissue is relatively avascular.

### DISCUSSION

Several points in the clinical history are worthy of emphasis. Very striking was the clinical picture, which was that of pulmonary disease with obstruction to blood flow through the lungs, rather than that of pelvic neoplasm. The polycythemia, together with hypertrophy of the right ventricle, was effective in maintaining compensation for about a year, but the last 6 months of life were characterized by a progressive failure of the right side of the heart.

The long duration of the disease is also interesting and indicates that the rate of growth of the lung tumor metastases was slow, a fatal termination occurring after 18 months of illness. Since there was no great change in the X-ray films during the last year of life it is probable that the lung metastases had been present for a long time before they had attained a size and number sufficient to produce the first symptoms. The microscopic appearance of the tumor is likewise that commonly associated with slow growth.

Another striking feature was the marked disproportion between

the extent of the disease as indicated by the X-ray studies of the lungs and the degree of incapacitation of the patient. Her general condition was better than would be thought possible, and the rate of decline was slower than that accompanying the commoner lung infections or metastatic tumor to the lungs in which the X-ray signs are so advanced. The absence of regression of the tumor tissue in the lungs under X-ray therapy is in conformity with what is known about the radiosensitivity of this type of tumor.

The age of the patient is also interesting. Most uterine sarcomas occur near the menopause, while this patient was only 36 years of age.

Williams<sup>11</sup> was among the first to show that malignant uterine tumors may be derived from smooth muscle. Mallory<sup>12, 13</sup> gave staining procedures by which muscle cell tumors can be identified on the basis of their fibrils. Characteristic fibrils are present in this tumor so there is no question as to its fundamental myomatous nature.

It is generally recognized that there is no sharp point of differentiation between benign and malignant muscle cell tumors. The myosarcomas in general have less stroma and are therefore softer and fleshier. They tend to be more vascular and are unencapsulated and poorly demarcated. They are cellular, with changes in the nuclei varying from little change to extreme anaplasia. In general the nuclei are shorter, more chromatic, uneven in size and shape, and show an increased number of mitotic figures. The loss of normal nuclear structure is accompanied by loss of the characteristic cytoplasmic fibrils. Anaplasia may be so marked that the fundamental nature of a tumor, whether it originated from muscle cells or fibrous stroma, cannot be determined.

In the routine microscopic examination of surgically removed uterine fibroids not infrequently cellular tumors exhibiting some of the cytological features associated with malignancy mentioned above are encountered. In the recorded literature this incidence varies from a small fraction of 1 per cent to over 10 per cent. Corscaden and Stout have recently discussed this situation<sup>14</sup> and have pointed out the error in diagnosis by those who find the higher figures. In these tumors the cytological changes are sometimes misleading since in a series of untreated fibroids, or in a series treated lightly by X-rays, no corresponding high incidence

of fatal uterine sarcoma is found. Uterine sarcomas in general autopsy series are relatively uncommon. Kimbrough<sup>15</sup> found that the 5 year survivals were three times as great in so-called sarcomatous degeneration of fibroids as in the primary uterine sarcomas. Any errors in diagnosis would most likely have been in the first group.

Although it is a common experience to find suspiciously sarcomatous regions in uterine fibroids, which are clinically permanently benign, the case here reported is an example of a much rarer condition in which a tumor composed of benign appearing cells proved to be clinically malignant.

Since the usual cytological changes are frequently misleading in the diagnosis of malignant uterine tumors, more reliable criteria have been sought. The mitotic figure count as an index of malignancy is accepted as of value by many. Evans<sup>16</sup> in 1920 pointed out that the clinical malignancy closely follows the number of mitotic figures. Kimbrough,<sup>15</sup> Handley and Howkins,<sup>17</sup> and others, have found this procedure of value. Meigs<sup>18</sup> states that malignancy must be suspected if more than 1 or 2 mitotic figures are seen in 10 to 25 high power fields. Casey<sup>19</sup> has studied the number of mitotic figures per 1000 tumor cells in a variety of neoplasms and has found that in general benign tumors have fewer than 4 and malignant tumors more than 4 mitotic figures per 1000 tumor cells. In this respect also the tumor in our case is unusual in that the number of mitotic figures is not correlated with the clinical malignancy. In the uterine tumors (2000 cells), lung metastases (1000 cells), and lymph node metastases (1000 cells) no mitotic figures were encountered (autopsy material). Accordingly, by this criterion, this case falls into the group of benign tumors, and this is in agreement with its general microscopic morphology. Yet its known clinical behavior was that of a malignant tumor.

The routes of metastasis in this case were undoubtedly by the veins from the uterus to the lungs and through lymphatics from the lungs to the tracheal lymph node. Several theories have been proposed to explain how cells so harmless appearing might metastasize. Thus Proper and Simpson<sup>20</sup> state: "They are probably really benign and metastasize as the result of the cells of a leiomyoma penetrating a blood vessel and producing another

tumor at the place where these cells become lodged." Van Rijssel<sup>21</sup> and Sitzenfrey<sup>22</sup> believe that benign appearing tumor cells enter blood vessels by pressure atrophy of the vessel wall.

*Nature of the Tumor:* Grossly there was little to indicate that the tumors in the uterus were malignant or capable of producing metastases. The appearance was that of a uterus enlarged by common fibroids. The tumors were firm, reddish gray, silky and whorled in most places, and on the whole shelled out readily. Nowhere in the uterine tumors or in the metastases was the tumor tissue friable or fleshy like a sarcoma. In retrospect one feature in the gross appearance may be of the greatest importance in recognizing this type of tumor. Although the uterine tumors separate easily from the tissue in which they are embedded, *some of them are seen to be not globular but elongated and to have a little tendency to interweaving or intertwining of adjacent tumors — in other words, a plexiform growth.* In this respect it resembles the case of Lahm.<sup>23</sup>

Microscopically, although the appearance was that of fibroleiomyoma, two points in examination of the uterine sections reveal the possibility of metastases. The tumor cells were capable of invading veins and they were able to infiltrate striated muscle. Neither of these features had been noted grossly. They indicate an unexpected potentiality of the cells and clarify the mechanism of the metastases.

Microscopic sections from this case have been seen by about twenty experienced pathologists. A few of them commented on the unusual degree of vascularity of some, but not all, areas in the uterine tumors. Others said that this was of a degree not uncommon in fibroids. Small blood vessels are numerous in some places and the tumor cells immediately adjacent appear more immature than those at a distance. This vascularity is less striking in the lung metastases, being entirely absent in large portions, and it is not seen at all in the lymph node metastasis. So it appears not to be an essential part of the growth.

Could the tumor be one of a primary vascular growth? The fact that the tumor cells immediately about blood vessels are shorter, appear younger, and that there is less collagen speaks for more rapid growth in these regions. The tumor cannot be proved to have a vascular origin. In fact, the presence of adventitial cells

separating the media of the blood vessels from tumor cells in certain areas is evidence against such a hypothesis. Furthermore, a perivascular proliferation of tumor cells is no proof of a vascular origin, but it may rather indicate a more favorable environment for growth in the form of better nutrition, a feature commonly seen in carcinomas and other tumors.

*Discussion of Nomenclature:* I have no strong convictions about the nomenclature of this type of tumor and have used the descriptive term "metastasizing fibroleiomyoma" at the insistence of some oncologists who saw the sections, and to strengthen the teaching points of the case. I have refrained from using the word benign. The incongruity of the term is apparent and I appreciate that the clinical outcome of the case resembled that of a malignant tumor.

In the nomenclature of malignant mesenchymal uterine tumors, by usage the term "sarcoma," with some qualifying term such as leio-, fibro-, mixed cell, undifferentiated, and so on, is widely accepted. This is true aside from the question of their primary origin from myometrium or from a previous fibromyoma. The term "myoma malignum" has been used by some writers for tumors in which the microscopic appearance suggested a malignant tumor but in which definite proof of malignancy was wanting, a position not infrequently met with in the routine examination of surgically removed fibroids. This same term is also used by others in a different sense to describe those instances in which a fibroleiomyoma becomes frankly malignant. The term "sarcoma myomatoides" has an even more diversified usage, having been used to describe tumors of each of the above three types. So none of these terms is entirely applicable to this case. Strong<sup>24</sup> has reviewed the problem of nomenclature in considerable detail.

Casting aside for the moment the term I have used, what name would be most suitable? I do not say that this is not a sarcoma, but if it is, that diagnosis must be made on other grounds than tissue morphology. Shall we say sarcoma of the uterus, as did Jacquin,<sup>25</sup> even if the histological appearance is benign? To do this is an admission that the diagnosis of sarcoma from biopsy material before the final clinical outcome is known is impossible in some instances.

As broad criteria of malignancy the familiar qualities of neoplasia, heterotopia and anaplasia present themselves. Of neoplasia

there can be no question in this case, but it is a property shared by benign and malignant tumors alike. Heterotopia was also present but there was no evidence of anaplasia. Under these circumstances the term myoma malignum might be considered more appropriate for this case than metastasizing fibroid, except that, as pointed out, this term is used by some in instances where cell anaplasia is present. In dealing with epithelial tumors the term "adenoma malignum" is used by some when there is neoplasia, a minimal degree of anaplasia, and no proof of heterotopia. It is useful in transmitting from the pathologist to the clinician the idea that the tumor is probably malignant but not so proved. In the present case the tumor was clinically malignant because of heterotopic muscle cells. McFarland<sup>26</sup> concludes that metastasis is the only proof of malignancy in dealing with muscle tumors. But benign fibroleiomyoma cells are capable of growing ectopically under special circumstances as shown by Brewer's<sup>27</sup> case in which cells from uterine fibroids, implanted into the abdominal wall, grew.

The problem as to nomenclature is not an important one since the question does not often arise.

#### REVIEW OF THE LITERATURE

*A. Metastasizing Fibromyomas of the Uterus:* A critical comparison of our case with those previously reported is extremely difficult because the earlier papers are not illustrated or are inadequately illustrated, histological descriptions are absent or sketchy, and there is considerable confusion in the nomenclature. Undoubtedly other cases have been overlooked, but the following 4 cases summarize those found in a fairly careful survey of the literature. Some of these cases are without description or illustration.

The 1st case reported was that of Krische<sup>28</sup> in 1889, in which fibromyomas of the uterus metastasized to many parts of the body. There was said to be abundant fibrous stroma. Orth<sup>29</sup> accepts this case as one of metastasizing fibroids without comment. Meyer,<sup>3</sup> however, refuses to accept this case, stating that its sarcomatous nature is firmly established.

The 2nd case of this type was reported by Langerhans<sup>30</sup> in 1893. It was that of a 60 year old woman who had multiple tumor

nodules in the uterus. One of these was predominantly smooth muscle, another was pure smooth muscle and the rest were fibromyomas. There were multiple pulmonary metastases composed of cells which were larger and more irregular than those in the uterus. There was, therefore, some change from normal smooth muscle structure (anaplasia) in the lung, the degree of which cannot be estimated because the paper is not illustrated. He called it a "myoma laevicellulare malignum."

The 3rd case on record is that of Minkowski,<sup>31</sup> who in 1901 at autopsy found metastases composed of adult smooth muscle but lacking all stroma, in the lungs, liver and muscles of a 43 year old woman who 2 years before had had a complete hysterectomy for a "fibromyoma" of the uterus. There are no illustrations or detailed descriptions, the case having been presented briefly before the medical society at Köln. He gave it the name of metastatic myoma.

The 4th case was that which Schlagenhauser<sup>32</sup> reported in 1902. A woman, 58 years of age, had multiple uterine tumors with metastases to the liver and lungs. Microscopically part of the uterine masses were typical fibromyoma while others were composed of muscle cells exclusively and had many large and small blood vessels, giving a cavernous appearance. There was no fibrous stroma. Some of the muscle cells showed anaplasia, being large and having multiple nuclei. In the metastases the vascular cavernous structure was not seen. The drawing which accompanied this report illustrates pure smooth muscle of a well differentiated type. He called his case "myoma telangiectodes."

Of these cases those of Langerhans and Schlagenhauser can be discarded from this group, since, by their own descriptions, the tumor cells showed a certain degree of anaplasia. Schlagenhauser was impressed by the vascularity of his tumor. His paper, alone, is illustrated. Minkowsky and Schlagenhauser thought that the absence of stroma (binding-substance) explained the malignant behavior of their tumors. Only the case of Krische had stroma.

In addition to these case reports of benign appearing metastasizing tumors of the uterus the literature contains references to such occurrences without presenting the details. Thus, Lockyer<sup>33</sup> states: "In common with other observers, I have examined the metastatic deposits from the lungs and pelvic cellular tissues of a

case in which there was a large myoma of the uterus, and where all the growths, primary and secondary, had the structure of an innocent-looking myoma. Unfortunately the specimen, which was removed by the late Stanley Boyd, had been lost, but the sections are preserved, and the lesson they teach suffices to obliterate any suspicion I may have had as to such a condition being a fact."

Also McFarland <sup>26</sup> in speaking of myomas in general, including those from the uterus, states, without giving details of such cases: "Histopathologic prognosis is fraught with difficulty. We have all seen, on the one hand, the tumor whose histological structure appeared to be that of an ordinary fibroid, accompanied by one or more metastases of identical appearance, and on the other, a tumor of the most suspicious appearance whose history has terminated with its removal."

Kaufmann,<sup>6</sup> also without citing a case, writes: "Malignant myomas produce metastases by the blood channels, possibly through the lymphatics also; therefore biologically, according to their growth, they are malignant, but histologically they are typical myomas, distinguished from sarcomatous myomas in that their muscle cells are more uniform and of higher development."

Not available to the writer is the volume in which Johnstone (according to Lockyer <sup>34</sup>) mentions 10 cases in which pulmonary and other metastases showed a structure of unstriped, benign appearing muscle tissue.

*B. Recurring Fibromyomas of the Uterus:* In discussions of this subject, the cases which von Franqué <sup>35</sup> reported in 1907 and which Jacquin <sup>25</sup> described in 1921 are usually included. They are examples, not of metastasizing but of recurring fibroleiomyomas. The case of Jacquin showed a microscopically benign recurrence 10 months after a hysterectomy for benign fibromyoma. The patient of von Franqué was even more interesting because of three recurrences over a period of 7 years. The tumors showed pure myoma with very little connective tissue, and areas of edema and of hyaline degeneration. The muscle of the vessel walls was not demarcated from tumor in some places.

More recently Neugebauer <sup>36</sup> in 1927 briefly reported an instance in which 6 years after supravaginal hysterectomy for benign myoma there were found three pelvic tumors entirely



separated from the amputation area and all showing the histological structures of simple benign myoma. This paper is without illustration or detailed histological report.

Christophorakos<sup>37</sup> described the case of a fibromyoma of the uterus in which there were three recurrences in 3 years, the first two of which appeared like benign myomas, while the third showed areas of pleomorphic sarcoma in addition to myoma. Matthews and Stier<sup>38</sup> saw a patient who died of a fourth recurrence and distant metastases about 10 years after a uterus with multiple fibromyomas was removed. The recurrences showed progressive deviation from normal, the first recurrence appearing benign and the last like a spindle celled sarcoma.

These cases of Christophorakos and of Matthews and Stier raise the question as to whether the other foregoing tumors would have changed their morphology to frank sarcoma if they had recurred again several times.

*C. Intravascular Uterine Fibromyomas:* In addition to the groups of metastasizing fibromyomas and recurring fibromyomas mentioned above, a third group of cases sheds light on this subject. This includes cases in which uterine fibromyomas extended into the regional blood or lymph vessels, yet failed to metastasize. They are of special interest in this connection because they may be illustrations of the early stages of the type of metastasis seen in the case here reported. Birch-Hirschfeld<sup>39</sup> (3 cases) and Meyer<sup>40</sup> described tumors with intralymphatic extensions. Von Franqué,<sup>35</sup> Hörmann,<sup>41</sup> Dürck,<sup>42</sup> van Rijssel<sup>21</sup> (2 cases), Seyler,<sup>43</sup> and Sitzenfrey<sup>22</sup> (3 cases) reported cases with intravenous growth. Lahm's<sup>23</sup> case was unique in showing both lymphatic and venous growth, although he saw changes at least suspicious of malignancy.

In this connection the report of Frank<sup>44</sup> should be kept in mind. He described 3 cases of plexiform growth within the lymphatics of the myometrium by a tissue which resembled endometrial stroma.

#### SUMMARY

A case is reported in which multiple tumor metastases to the lungs from the uterus led to chronic obstruction to blood flow through the lungs, polycythemia, enlargement of the right side of the heart, and finally to heart failure. The uterine tumors micro-

scopically appeared to be benign fibromyomas with areas that were quite vascular. The lung and tracheal lymph node metastases appeared even more mature and benign. In the absence of changes in color and consistence, local invasion of veins or rapid growth, uterine tumors which resemble fibroids but have a little tendency to plexiform growth should be considered potentially malignant. No new microscopic criteria have been discovered by which the malignancy of such cases can be recognized in the future. The mitotic figure count on postmortem specimens was non-informing as to the malignancy. The tumor in our case was histologically benign appearing, but clinically malignant. Such cases are much rarer than those in which histologically malignant appearing uterine tumors (cellular myomas) prove to be clinically benign. Four cases of metastasizing uterine fibromyomas reported previously, 2 of which, however, showed signs of malignancy, are briefly summarized.

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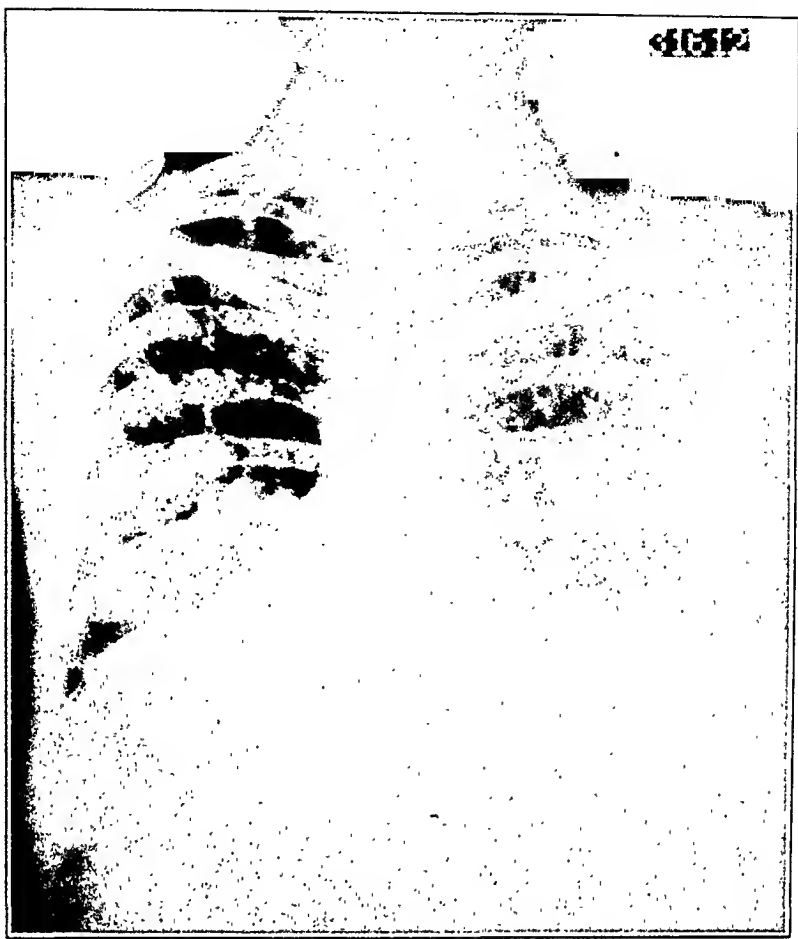
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## DESCRIPTION OF PLATES

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### PLATE 21

- FIG. 1. X-ray of chest made about a week before death. A similar degree of involvement was present a year earlier. The picture is not like that usually produced by tumor metastases to the lungs.
- FIG. 2. X-ray of the excised inflated lungs. The solid and the cystic nodules can both be seen, the latter particularly at the margin. The heart is still attached. The large tumor metastasis is in the right lung.



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Steiner

Metastasizing Fibroleiomyoma of Uterus

PLATE 22

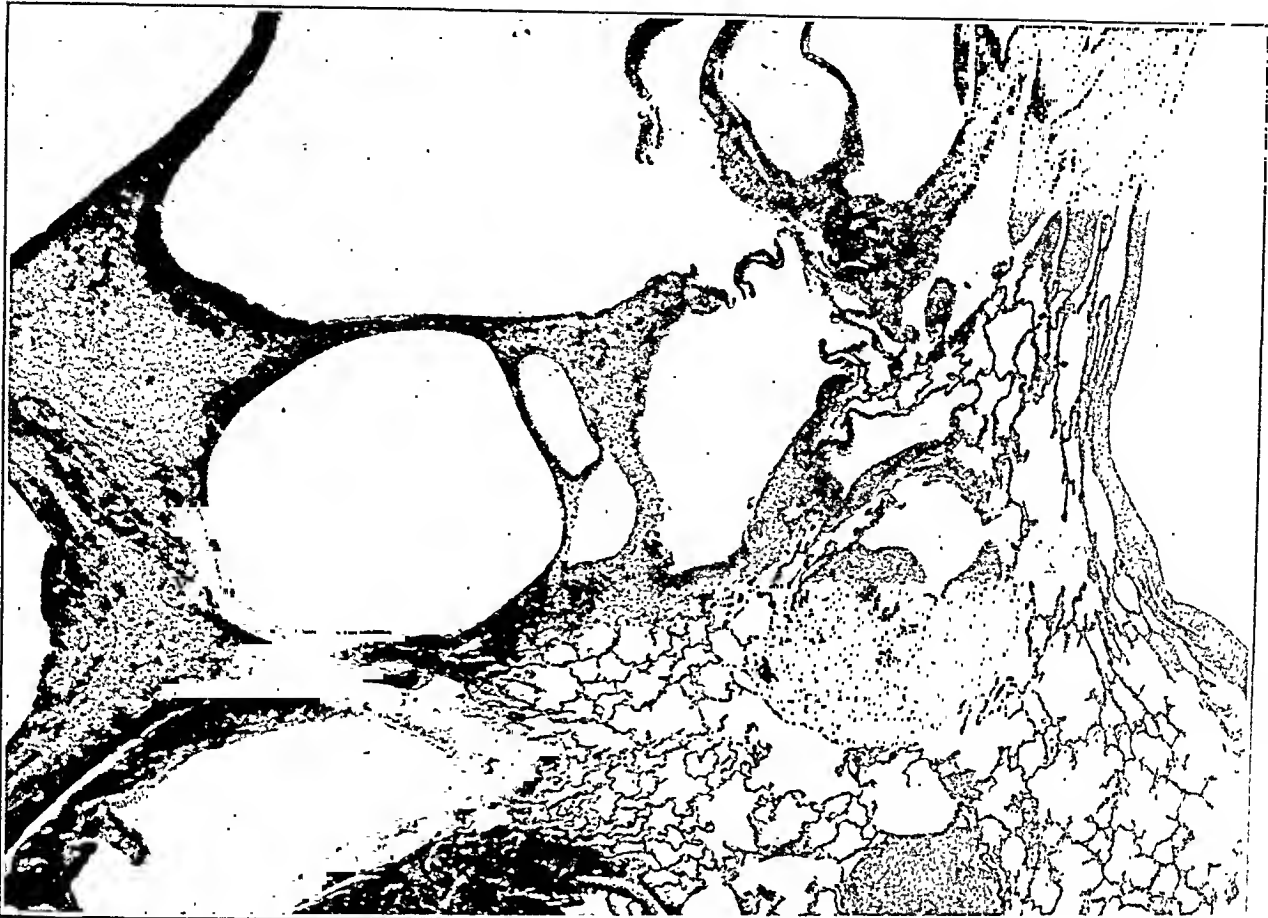
- FIG. 3. Posterior view of the lungs. The largest tumor mass is in the right lung. Pedunculated metastases can be seen at the margin.
- FIG. 4. Anterior view of the lungs. Some tumor nodules are solid and others are air-containing with walls containing fibromyomatous tissue.
- FIG. 5. Uterus. From below upward are seen the vagina, cervical canal and uterine cavity. Numerous tumor nodules occupy the anterior and upper uterine walls, and several nodules extend out into the right broad ligament.



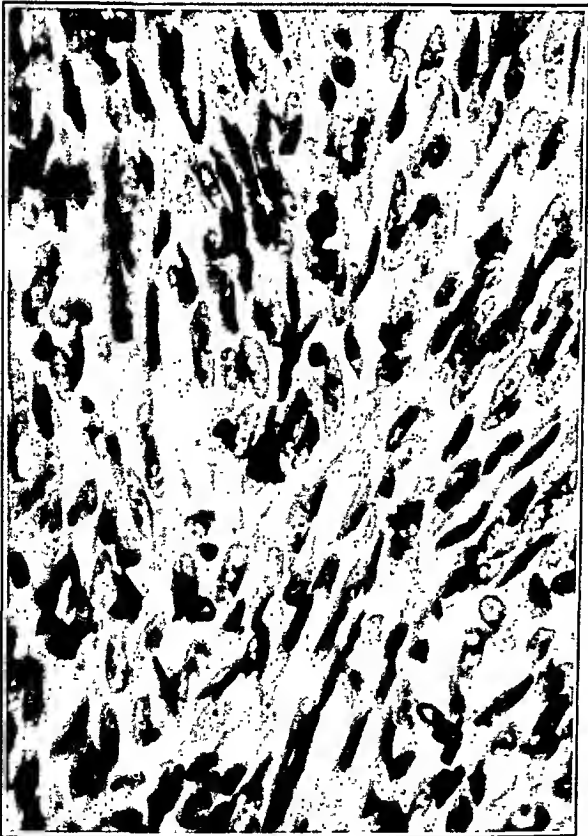


PLATE 23

- FIG. 6. Section showing metastases to lung. The walls of the cystic tumor nodules are composed of smooth muscle lined by epithelium. A small, solid tumor metastasis is also shown. Only small areas of aerated lung resembling that illustrated are present in both lungs.  $\times 10$ .
- FIG. 7. Section showing the myomatous nature of the metastases to the lung. Hematoxylin-eosin stain.  $\times 650$ .
- FIG. 8. Section of metastasis to lung illustrating myoglia fibrils and myofibrils in cross and longitudinal section. Phosphotungstic acid hematoxylin stain.  $\times 2600$ .



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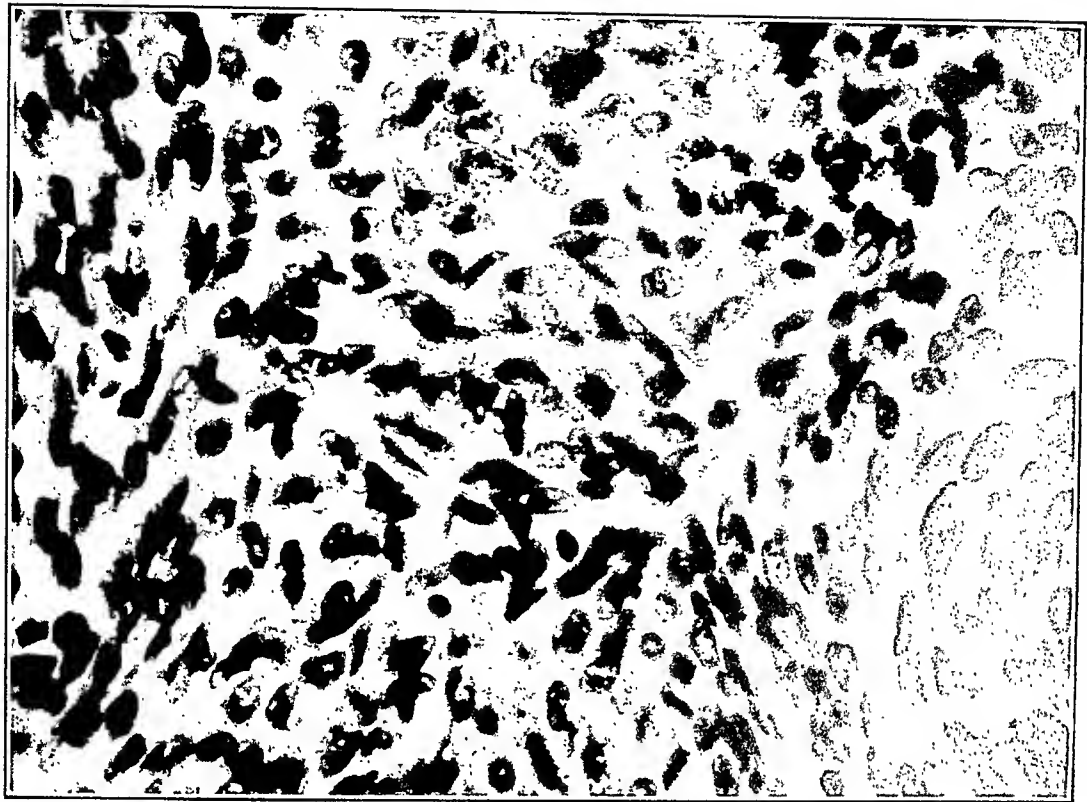


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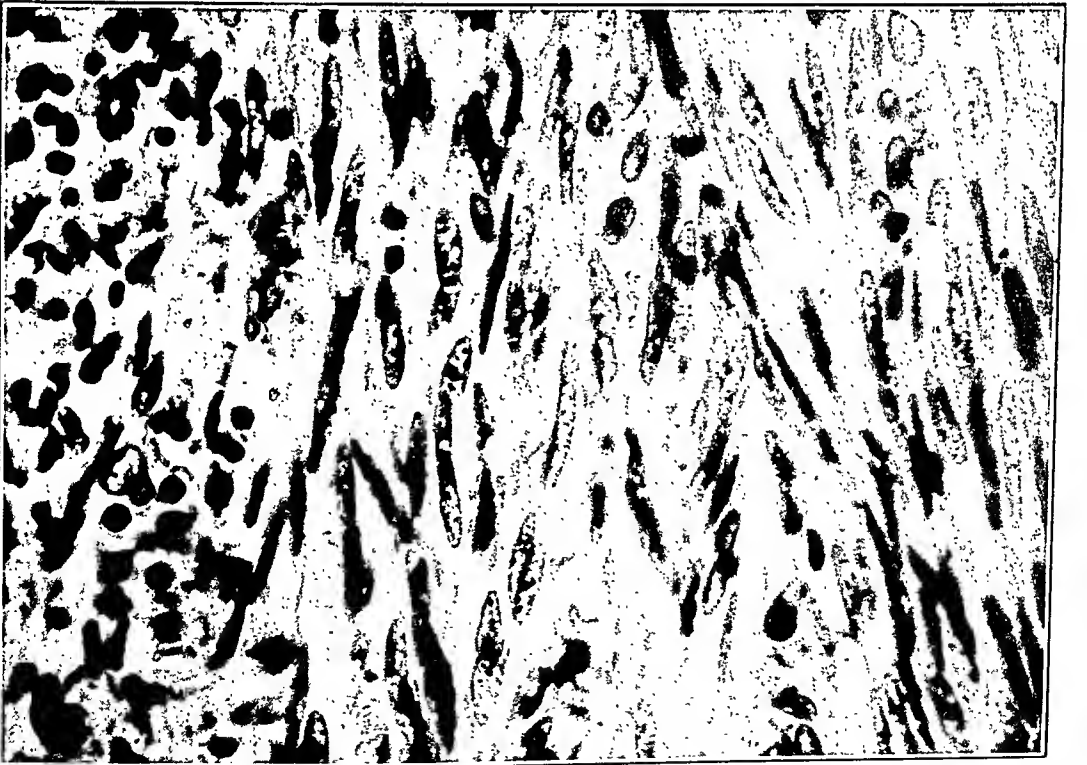
PLATE 24

FIG. 9. Section of a cellular area in the uterine tumor. Many of the cells are transected. Areas more suspicious than this were not seen. Hematoxylin-eosin stain.  $\times 700$ .

FIG. 10. Metastasis in the peripheral sinus of a tracheal lymph node. The fully differentiated myomatous nature of the tumor is easily seen.  $\times 550$ .



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Steiner

Metastasizing Fibroleiomyoma of Uterus



PRIMARY HYPERPARATHYROIDISM WITH EXTENSIVE RENAL  
CALCIFICATION AND SECONDARY HYPERPLASIA  
OF THE PARATHYROIDS \*

REPORT OF A CASE

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That hyperparathyroidism may produce renal insufficiency through renal calcinosis and calculus formation, and that renal insufficiency of varied etiology may be associated with hyperplasia of the parathyroid glands is now well established. Albright and Bloomberg <sup>1</sup> conclude that hyperparathyroidism is such a frequent cause of renal stone formation that its presence must be ruled in or out in every case of this disease and cite 11 cases out of 23 proved cases of hyperparathyroidism where the presence of renal stones was the only clue that led to the diagnosis of the underlying condition. Ettinger and Magendantz <sup>2</sup> reported a case with decreased urea clearance and phenolsulphonaphthalein excretion but uncomplicated by nitrogen retention, where evidence of extensive calcification of the kidneys was immediately evident on X-ray examination. In discussing the renal complications of hyperparathyroidism, Albright, Baird, Cope and Bloomberg <sup>3</sup> consider the renal lesions as divisible into three groups: (1) those (type I) associated with a pyelonephritis secondary to the formation of calcium phosphate stones in the renal pelvis; (2) those (type III) presenting calcium deposits in the kidney parenchyma as well as in other organs, but without chronic renal change and associated with acute parathyroid poisoning with anuria and death from undetermined cause in a few days; and (3) a third group (type II) differing from type I in that the calcium deposits are in the parenchyma of the kidney, which may be the only organ involved, rather than in the kidney pelvis, and from type III in that the kidneys show long standing changes simulating both chronic glomerular and vascular nephritis.

Over 30 years ago MacCallum <sup>4</sup> first reported parathyroid en-

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largement associated with nephritis. Bergstrand<sup>5</sup> in 1921 more particularly called attention to the relation between renal function and parathyroid enlargement. More recently sufficient evidence has been accumulated from experiments and autopsies to conclude that parathyroid enlargement is a relatively constant finding in renal insufficiency. Thus, Pappenheimer and Wilens<sup>6</sup> conclude from an analysis of the weight of the parathyroids in 27 nephritics and 72 miscellaneous cases showing normal kidneys that the mean weight of the parathyroids in various types of chronic renal disease exceeds that of the non-nephritic cases by from 50 to more than 100 per cent, that usually three or four of the glands share in the enlargement, and the weight increase is roughly proportional to the severity and extent of the renal lesions and to the intensity of the clinical signs of renal insufficiency. Castleman and Mallory<sup>7</sup> report 29 cases of "secondary" hyperplasia associated with chronic renal insufficiency of various grades, offering certain histological criteria they consider specific of secondary hyperplasia. Their 29 cases presented varying degrees of enlargement of the parathyroids and even when the enlargement was limited to a single gland, histological examination always revealed evident hyperplasia in the other glands also. Furthermore, Jarrett, Peters and Pappenheimer,<sup>8</sup> and Pappenheimer<sup>9</sup> have shown in the rat that reduction of functional renal substance leads to a decided increase in size of the parathyroids.

In the light of these observations we wish to present for consideration a case\* we believe may be interpreted as one of classical primary hyperparathyroidism associated with extensive calcification of a horseshoe kidney (Albright's type II). Removal of the parathyroid tumor was followed by dramatic improvement, a return to normal of the blood chemistry, and healing of the bone lesions. The extensive renal calcification persisted, however, and over the space of a few years hypertension and marked renal insufficiency developed, resulting in enlargement of the remaining three parathyroid glands and death in uremia.

\* The case is 1 of 4 reported by Gutman, Swenson and Parsons<sup>10</sup> in 1934 (Case 1) and their article is illustrated by X-rays of bones and of the abdomen demonstrating calcification of the horseshoe kidney. It includes a table of balance studies. Certain of the chemical data have been further reported by Gutman, Tyson and Gutman<sup>11</sup> in 1936 (Case 1). However, we shall include a very brief clinical summary and complete the clinical and chemical data.

## REPORT OF CASE

*Clinical History* \*: A 34 year old American salesman was first seen at the Presbyterian Hospital in July, 1933, complaining of pain in the right hip for 9 months and of increasing weakness for about 2 years. There was no family history of disease of the bones or childhood history of rickets or late walking. For the past 2 to 3 years he had been losing weight and strength and had noted thirst and polyuria with nocturia (3 to 4 times). There was no history of hematuria, oliguria, dysuria, hypertension, acute nephritis, sore throat, tonsillitis or scarlet fever.

One year before entry the patient fractured his right humerus when throwing a ball. There was no history of direct trauma. X-ray studies revealed a cyst in the region of the fracture. The remainder of the skeleton was not examined. The fracture healed in 8 weeks, the patient being given calcium, cod liver oil, and ultraviolet therapy. During this time he lost 30 pounds. He was given iron intramuscularly, liver extract, and iron by mouth, and treatment with cod liver oil and calcium lactate, supplemented by viosterol, was continued. His general health improved and he gained 20 pounds and was able to return to work.

Six months before entry his urine was said to have contained albumin. About this time slight trauma to the right shin was followed by swelling, not especially tender or painful, which on subsiding in about 2 weeks left a hard, smooth, irregular bony prominence that persisted unchanged up to the time of admission.

Three months prior to admission an X-ray of the hip because of pain in that region revealed the presence of three cysts and the patient found that he required a hat  $\frac{1}{4}$  size larger than he had worn during the previous 14 years. He had lost 30 pounds. About this time he began to vomit 3 to 4 times per week, usually in the morning. Although nausea and occasionally vomiting was associated with the cod liver oil, he continued taking it with calcium lactate after finding that omission resulted in exacerbation of his symptoms.

On admission to the hospital physical examination revealed: temperature 99.4, pulse 88, respiration 22, blood pressure 150/90 mm. Hg. The heart was not enlarged. The urine showed a very faint to faint trace of albumin but no Bence-Jones protein. The specific gravity was 1.006-1.012 and glucose 0. Microscopic examination on five occasions was negative; twice there were reported white blood cells, and once "casts and crystals." The Wassermann reaction was negative. The red blood cells were 3,760,000, and the white blood cells 15,200. A differential count was normal. The serum calcium was 15.7 mg. per cent, phosphorus 2.6 mg. per cent, phosphatase 33.9 Bodansky units per cent, serum protein 6.5 mg. per cent, and the blood urea nitrogen 35 mg. per cent. Roentgenograms revealed that practically all the bones contained multilocular cystic areas of decreased density. The skull presented a characteristic finely granular appearance, and there was seen a horseshoe kidney containing many areas of calcification. Metabolic studies showed definitely increased urinary excretion of calcium and phosphorus. After balance studies the patient was kept on a high calcium diet.

\* I am indebted to Dr. A. B. Gutman and to Dr. W. B. Parsons for the clinical data and permission to report this case, and to Dr. A. M. Pappenheimer for help in the preparation of this material.



He was operated upon on Aug. 17, 1933, and an adenoma of the right lower parathyroid was discovered. The gland was removed and was found to weigh 3.5 gm. Gross and histological descriptions of this adenoma will be found below with the description of the remaining three parathyroids. Following operation he had slight transient hypoparathyroidism, readily controlled with

TABLE I  
*Data on Blood Pressure and Blood Chemistry*

Date	Blood Pressure	Calcium	Phosphorus	Phosphatase	Non-protein nitrogen
	<i>mm. Hg.</i>	<i>mg. %</i>	<i>mg. %</i>	<i>mg. %</i>	<i>mg. %</i>
7/10/33	150/90				
7/11/33	135/90				
7/14/33		15.7	2.7	34.9	Urea 35
7/15/33	134/80				
7/24/33	130/90				
7/29/33	130/90				
8/1/33		15.4	2.6	34.8	39
8/15/33		15.8	3.1	28.3	40
8/17/33	* 118/- * 105/- * 165/- * 135/-				
8/18/33		9.7	2.4	32.1	47
8/20/33		9.2		27.9	39
8/21/33		8.8	1.9	29.9	
8/23/33		8.7	2.1	36.2	25
8/28/33		8.1	2.7	45.6	
10/6/33		9.4	4.2	16.6	51
12/29/33		10.5	4.0	7.9	60
3/16/34		10.4	4.0	6.1	60
8/7/34		10.2	3.0	4.2	44
7/23/35		10.3	2.9	4.5	50
1/26/37		9.7	3.5	4.2	54
6/22/37	210/120	9.8	3.3	4.6	66
9/20/37	200/120	9.2	3.3	5.4	79
2/9/38	240/150	10.5	6.0	5.4	110
2/10/38					107
2/12/38	240/130	9.6	5.2	4.4	107
2/15/38					95
2/18/38	170/110	9.2	4.6	3.8	125

\* Systolic pressures at start and at 10 minute intervals during operation for removal of the parathyroid tumor.

calcium lactate. The blood calcium returned to normal and remained normal, and over a period of months the serum phosphatase approached normal. The bone lesions healed and the patient improved and returned to work. He was followed intermittently in the out patient department and was seen again 4 years after operation complaining of loss of weight, occipital headache and nocturia. The blood pressure was 210/120 mm. Hg. The heart was slightly enlarged to the left, the apex impulse not diffuse or heaving. The non-protein nitrogen was 66 mg. per cent. Three months later the blood pressure was

200/120, the apical impulse seemed more diffuse, and the non-protein nitrogen was 79 mg. per cent.

He reentered the hospital in February, 1938. The temperature was 98.6, the pulse 120, respiration 32, and the blood pressure 240/150 mm. Hg. He was irrational and had Cheyne-Stokes respirations. There was no edema. Narrowing and obliteration of the vessels of the fundi with small hemorrhages were seen. The breath was not uremic. Dullness and sticky râles were present at the base of the left lung posteriorly. The heart was diffusely and greatly enlarged to the left, with heaving apex impulse, rapid rate, A<sub>2</sub> greater than P<sub>2</sub>, hollow blowing apical systolic murmur and gallop rhythm. He had a convulsion lasting half an hour characterized by clonic spasm in the extremities and jaws, with loss of consciousness and incontinence of stool and urine. The hemoglobin was 65 per cent, red blood cells 3,140,000, white blood cells 14,800, and polymorphonuclears 87 per cent. The guaiac test on a stool was 2+. The specific gravity of the urine was 1.008-1.011, albumin + to +++, glucose 0, and there were 2 to 4 red blood cells on occasional specimens but no casts. The serum phosphatase showed 5.4 Bodansky units per cent, inorganic phosphorus 6 mg. per cent, calcium 10.5 mg. per cent, non-protein nitrogen 110 mg. per cent, cholesterol 258 mg. per cent, protein 5.7 mg. per cent, albumin 3.9 mg. per cent, and globulin 1.8 mg. per cent. An X-ray revealed the outline of a horseshoe kidney containing large amounts of calcium, but when compared with the previous film there seemed to be no change in the extent or density of these calcium shadows.

The patient developed bronchopneumonia in both lungs with rapid respiration, became comatose and died.

*Clinical Diagnoses:* Healed hyperparathyroidism, calcification of horseshoe kidney, uremia, cardiac hypertrophy, secondary hypertension, and bronchopneumonia.

#### ABSTRACT OF AUTOPSY \*

Permission could not be obtained to remove the calvarium. For purposes of completeness and brevity it may be said that the spleen, liver, gall bladder, pancreas, adrenals, bladder, prostate, seminal vesicles, right testis, alimentary tract, thyroid, thymus, superior pubic ramus, vertebrae and cortex of the left femur appeared normal in gross. The significant findings were as follows:

*Heart:* Weight 480 gm. The left ventricle measures 2 cm., the right 5 to 6 mm. in thickness. There is definite hypertrophy of the left ventricle. The valves are normal. The coronary arteries show slight atheroma without calcification. There is no gross evidence of fibrosis.

*Lungs:* The right lung weighs 700 gm. It is somewhat boggy and on section the parenchyma is quite moist with numerous, mottled, darker red firm areas in no constant relation to the

\* No. 12711.

bronchioles. The left lung weighs 550 gm. and is essentially similar to the right. The lower lobe shows the more marked changes, being firmer and darker red.

*Kidneys:* There is present a horseshoe kidney which is removed with the aorta and is not weighed. The right portion measures 10.5 by 4 by 2 cm., the left, 10 by 4 by 2 cm. The isthmus bridging the vertebral column joins the lower poles and measures 3 by 2 by 5 cm. The pelvis on each side is dilated but shows no evidence of infection. No stones are present. The ureters are only slightly dilated, the right measuring 1 cm. in internal circumference, the left 13 mm. The capsule strips with moderate difficulty. The renal surface is extremely irregular and finely stippled with small, usually spherical white prominences which are surrounded by injected renal tissue. These small prominences measure 1 mm. in greatest diameter. Section of the kidney, however, particularly as the knife passes through the medulla, gives the impression of an extreme degree of calcification, a granular gritty sensation being transmitted. The kidney cortex is defined with difficulty and measures at the most 3 mm. The radial and glomerular structures are clearly seen. There is abundant deposition of calcium in the medulla. These grayish yellow, granular calcium deposits cause the pyramids to contrast sharply with the cortex. There are small cystic areas to be seen in the cortex. Dilated calices extend almost to the renal surface. A stain of a thin section (1 to 2 mm.) with silver nitrate in the dark, according to Kesten's technique<sup>12</sup> for the demonstration of calcium in gross material, and clearing the specimen by Spalteholz' method, reveals by transmitted light a most striking calcification of the renal pyramids. Calcium determinations\* disclose an average for three specimens of 2122 mg. of calcium per 100 gm. of wet kidney; the specimens are from different portions of the kidney and contain respectively 1715, 2691 and 1962 mg. of calcium per 100 gm. (average from 9 normal kidneys, (12 determinations) 9.3 mg. calcium per 100 gm.).

*Testes:* The left testis is small, approximately one-third the size of the right. Its tunica is not thickened and the tubules do not string out readily. The epididymis is small and firm.

*Parathyroids:* The specimen removed at operation in 1933 was

\* The methods used by Donohue, Spingarn and Pappenheimer<sup>13</sup> were employed for all determinations of tissue calcium.

found on the right side, arising from and partially embedded in the posterior aspect of the lower part of the right lobe of the thyroid. It was entirely surrounded by an extremely thin capsule and weighed 3.5 gm. The gland was oval and very soft. It measured 2.5 by 1.6 by 1 cm. The freshly cut surface was smooth and fairly homogeneous, the gland appearing divided and one-half slightly more yellow than the other. A few tiny petechial hemorrhages were seen within this soft smooth tissue; no trabeculae could be made out and there were no gross areas of degeneration.

Three parathyroids are located at autopsy. These are the left upper, weighing 177 mg.; the left lower, 129 mg.; and the right upper, 93 mg.; a total for the three glands of 399 mg. They are definitely enlarged but not unusual in color or consistence.

*Ribs:* A fusiform swelling of the fifth rib on the left is noted in the anterior axillary line. On cross section this presents an unusually thick but symmetrical cortex and prominent trabeculae. A similar though larger swelling is found anteriorly on the right eighth rib. A short distance from the head of the second rib there arises a "collar button" shaped prominence approximately 1.5 cm. in length which, projecting into the right pleural cavity, produces a dimple-like depression of the lung over it. This mass is firm and hard and on section reveals no cystic areas.

#### MICROSCOPIC EXAMINATION

Sections of heart, aorta, lungs, liver, spleen, pancreas, adrenal, kidney, bladder, prostate, testes, stomach, intestine, colon, thyroid, parathyroids, thymus, femoral marrow, vertebra, second and third rib (right) with "collar button" projection, fifth rib (left), eighth rib (right), superior pubic ramus and cortex of the left femur were studied histologically.

It may be mentioned briefly that there is histological evidence of myocardial hypertrophy with a moderate diffuse fibrosis, moderate arteriosclerosis, lobular pneumonia, acute splenic tumor, atrophy of the left testis with absence of spermatogenesis, and a rather striking hyperplasia of the cells of Leydig in each testis. No calcium deposits are found in any of the viscera except in the horseshoe kidney.

Deserving of more consideration are certain vascular changes,

the renal lesions, the condition of the bones examined and the parathyroids.

*Vascular Lesions:* The arterioles of the pancreas, spleen and adrenals are markedly sclerotic, some having undergone necrosis. In addition there is extreme arterial and arteriolar sclerosis in the kidneys which may well in part explain the renal atrophy (Fig. 5). Arterioles, particularly in the adrenal and periadrenal tissues, are frequently converted into hyaline masses with no evidence of a lumen (Figs. 6 and 7). Occasionally the vessel wall is widened, the intima is markedly thickened and darker areas with large yellowish green "glassy" deposits comprise the greater portion of the intima. Small thrombi are encountered occasionally. Numerous medium sized arteries of the pancreas are the site of an obliterative process that has frequently progressed to complete occlusion of the lumen; occasionally there has occurred recanalization and central hemosiderin deposition. Elastic tissue stains reveal fragmentation or complete destruction of the elastic components of the arterial wall. Similar vascular lesions are seen in the spleen. The arteriolar lesions in the kidney are even more striking and involve branches of all sizes. The basophilic change in the intima is particularly striking and the vessels appear larger, as though the thickening intima, as it encroached upon the lumen, pushed outward also the media and surrounding renal tissues. Many of the afferent arterioles are converted into hyaline masses and hyalinization is frequently seen in the thickened intima of larger arteries. Several arteries contain thrombi and others show evidence of recanalization. In places in the area of scarring calcium is deposited in the walls of the arteries.

*Kidneys:* Various sections disclose marked and consistent changes. The glomeruli are in many instances normal. The capsules of Bowman are delicate, the spaces empty and the glomerular tufts well defined. There are, however, a great many completely hyalinized glomeruli, while others show thickening of Bowman's capsule, and partial glomerular hyalinization. Occasional glomerular capillaries either contain thrombi or have become necrotic. Many of the convoluted tubules are normal; others contain inspissated casts frequently calcified. There are irregularly defined areas of tubular dilatation. A few small areas of calcification are found in the interstitium of the cortex with slight dilatation of the

distal convoluted tubules. The most striking alteration, however, is seen in the region of the collecting tubules where there are large masses of calcium and complete destruction of tubules in many areas. Calcium extends as broad plaques through the pyramids replacing tubular and interstitial elements. Throughout the interstitial tissue of the cortex and medulla are numerous aggregates of lymphocytes.

*Parathyroids:* The tumor removed from the region of the right lower lobe of the thyroid is apparently well encapsulated by connective tissue, portions of which are hyalinized and contain hemosiderin. It is composed in greater part of extremely large cells grouped in small, oval or rounded nests of 5 to 20 cells enclosed by very slender connective tissue septums (Fig. 2). Strikingly apparent are stages of transition suggesting a relation between the four cell types. These are: (1) large cells, measuring 25 to 35 microns, oval and spherical, and frequently polyhedral in outline. The nuclei measure 15 to 25 microns, are usually spherical and centrally located but vary considerably in shape; they are frequently vesicular and contain a rich and irregularly arranged chromatin network. The nucleolus is prominent and eccentrically located, occasionally staining with eosin. The cytoplasm is composed of pinkish granular material, varying considerably in amount and frequently seen only at the cell periphery, leaving a clear halo about the nucleus; at other times the granules are more compact and the halo is absent. This type of cell is the most numerous. It is probably a neoplastic derivative of the chief cell or of the transition water clear cell. (2) Rarely, water clear cells are seen, though many of the cells considered to be derivatives of the chief cell have lost almost all cytoplasmic granularity and resemble closely the water clear cell; these are somewhat smaller than the chief cell, measuring 15 to 25 microns, the vesicular nuclei measuring 5 to 8 microns. (3) Nests of brilliant red polyhedral cells with compact granular cytoplasm and hyperchromatic nuclei are present. These cells measure 25 to 35 microns, the nuclei 10 microns. (4) In the midst of the tumor is a small island composed of smaller brilliant red cells with intensely hyperchromatic pyknotic nuclei; these cells measure 7 to 15 microns, their nuclei 5 to 7 microns. Occasionally various cell types form syncytia within the nests, at other times a single

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cell appears to contain 2 to 3 nuclei. Mitoses are infrequently seen.

At the periphery of the tumor and lying just beneath the capsule is a rim of normal parathyroid tissue. Chief cells measuring 7 to 8 microns are predominant; their nuclei, usually vesicular, measure 5 microns. Numerous water clear elements and transitional forms are present. There are short columns of small, dark oxyphilic cells measuring 6 to 7 microns, with intensely hyperchromatic nuclei 3 to 4 microns in diameter.

The histology of the other three glands is essentially similar. They are vascular and hyperplastic, with no fat and only a moderate amount of stroma. The chief cell measuring 7 to 8 microns is predominant though many water clear and transitional forms are seen (Fig. 3). In several areas the chief cells are arranged in cords; more frequently they form closely packed groups. Some of the nuclei are hyperchromatic; others are vesicular. They measure 3 to 5 microns. The cytoplasm is finely granular, staining either blue or bluish pink. In many cells there is a perinuclear halo with transition to cells with completely water clear cytoplasm. The water clear cells are usually somewhat larger, measuring 8 to 12 microns with vesicular or hyperchromatic nuclei measuring 6 to 7 microns. Oxyphils with very finely granular cytoplasm are seen either isolated or in small groups. They are polyhedral in outline and measure 10 to 13 microns; their nuclei are hyperchromatic and measure 4 to 5 microns in diameter. In the right upper gland is a nest of approximately 50 similar cells. Occasionally these cells also show a perinuclear halo. In each gland are follicles, some of moderate size and each containing a homogeneous, eosinophilic colloid-like substance.

The glands are otherwise of interest in that at the periphery of both the left upper and left lower glands thymic tissue is in intimate relation with the capsules.

*Ribs:* The second and third (right), with projecting "collar button" nodule, appear normal. The cortex is not thickened and the trabeculae are slender. There is no osteoclastic activity or fibrosis of the marrow. A moderate amount of hematopoietic tissue is embedded in the marrow fat. The projecting mass is composed of osteoid tissue encapsulated by periosteum continuous with that of the second and third ribs.

Eighth (right) and fifth (left) ribs, through fusiform swellings show the cortices to be irregularly and considerably thickened. The trabeculae at the periphery are broadened, short and somewhat irregular, those in the center are slender and more normal in appearance. The marrow cavity consists largely of fat and there is no fibrosis.

*Vertebrae:* The osseous elements are normal. There is no fibrosis of the marrow and hematopoiesis is active.

*Pelvis:* Sections reveal some thickening of the cortex; the trabeculae at the periphery are broad and similar to those seen in the ribs. There are small areas of fibrosis in the marrow and a moderate number of osteoclasts with some evidence of osteoblastic and osteoclastic activity.

## DISCUSSION

There are three problems in this case that merit discussion. These are the mechanisms that resulted in the nephrocalcinosis, the etiology of the hypertension and the vascular alterations, and the nature of the parathyroid enlargement.

Donohue, Spingarn and Pappenheimer<sup>13</sup> have shown an increase in the calcium content of the residual renal tissue in rats rendered uremic by experimental reduction of kidney substance, but in our case extensive renal calcification occurred many months before nitrogen retention appeared. The only evidence of renal damage when the patient was first observed was the presence of slight albuminuria and a failure to concentrate the urine to a specific gravity greater than 1.012. This may have been due to the diuresis accompanying hyperparathyroidism. Suggested in the X-ray and strikingly apparent in the specimen is the concentration of calcium in the pyramids, whereas the cortical arches and columns of Bertini are relatively unaffected. Apparently the renal changes in this case are concerned primarily with the formation and inspissation of calcium casts in the collecting tubules. Lubarsch (Albright and Bloomberg<sup>1</sup>) describes calcium phosphate casts seen in hyperparathyroidism. Albright and Bloomberg suggest that the factors governing nephrocalcinosis are similar to those concerned with the precipitation of calcium phosphate *in vitro*, i.e. the pH of the solvent and the concentration of calcium and phosphorus. The formation of stones has been recently discussed by Keyser.<sup>14</sup>

Although the mechanism of parathyroid hormone action is controversial, certain effects are known. Relative to nephrocalcinosis Chown, Lee and Teal<sup>15</sup> have experimentally shown extensive calcification of the kidneys following parathormone administration. Morgan and Samisch<sup>16</sup> and Olsen<sup>17</sup> have demonstrated chemically an increase of the calcium content of the kidney after injections of parathormone. We have produced in a rat by intraperitoneal injection an increase of renal calcium comparable to that found in our case. Clinically hyperparathyroidism is associated with an increased calcium and phosphorus excretion in the urine. Therapeutically and experimentally in man and in animals parathormone administrations increase the urinary output of calcium and phosphorus.

In the case reported here the pH of the urine was not known at the time the calcium deposition in the collecting tubules took place, nor is there a history of pyelitis or of renal calculi. There was evidence of a high urinary calcium and phosphorus excretion, as shown by balance studies, and this was probably increased by long continued calcium, viosterol and ultraviolet ray therapy. It seems fair to conclude that renal calcinosis in this case resulted from an increased concentration of calcium and phosphorus in the glomerular filtrate, brought about by excessive parathyroid activity. Calcification of necrotic renal parenchyma is present to some extent but the nephrocalcinosis found appears to differ only in the site of calcium deposition from nephrolithiasis associated with hyperparathyroidism. In the case discussed here the deposition of calcium in the urinary excretory passages has occurred at a point nearer to Bowman's space than the renal pelvis, namely in the collecting tubules.

The second problem is the interpretation of the vascular lesions. It may be assumed : (1) either that there was present a coincidental, essential or primary hypertension with generalized arteriolar sclerosis and necrosis responsible in part for the rapid development of renal insufficiency; or (2) that the generalized vascular lesions are secondary to the renal alterations. The patient was seen occasionally in the out patient department, but it was not until almost 4 years after the operation that symptoms of hypertension appeared; during this interval there are no records of the blood pressure. Near the end of this 4 year period definite hypertension

was evident. There followed rapid progression of the hypertensive cardiovascular renal disease.

It is not believed that so-called primary hypertension can be ruled out as a complication. The hypertension apparently did not appear until almost 4 years after extensive nephrocalcinosis had been demonstrated by X-ray; hence the generalized arteriolar sclerosis and necrosis may perhaps be considered as indicative of the malignant phase of a primary hypertension. The age of the patient and the rapid progress of the disease may also be considered in favor of primary hypertension.

Primary or essential hypertension is a definite clinical-pathological entity of obscure etiology. The case here reported is complicated by a preceding nephrocalcinosis that may have had some influence on the development of the vascular lesions. For this reason further discussion of a possible relationship seems pertinent.

Without the presence of a coincidental primary hypertension it must be assumed that the deposition of calcium resulted in atrophy of the renal parenchyma. Such atrophy has long been associated with hypertension but is not found to be the primary factor in the generalized arteriolar sclerosis and necrosis present in this case. Goldblatt<sup>19</sup> has produced in dogs diffuse arteriolar hyalinization and necrosis similar to that seen in this case and usually considered to be characteristic of the malignant phase of primary hypertension. He points out that, experimentally, at least two factors are necessary. These are: (1) elevation of blood pressure (mechanical factor); and (2) renal ischemia (humeral factor). Previously<sup>18</sup> he had shown the importance of renal ischemia induced by clamping the renal artery in the production of the hypertensive state whether one or both kidneys were rendered ischemic. It was by severely constricting the renal arteries that he produced diffuse arteriolar hyalinization and necrosis; such vascular lesions were not found in the kidney, presumably because the mechanical factor was not present.

The massive calcification of the renal pyramids may have resulted in renal atrophy and sclerosis of the afferent arterioles of certain of the glomeruli with resultant increased intrarenal vascular tension. Furthermore, the deposition of the calcium near branches of the renal artery may have compressed them and produced ischemia in certain areas of the renal parenchyma. This

latter occurrence might have given further impetus to the hypertension; this possibility, however, cannot be proved. It is hazardous to assume that the decreased blood supply of an atrophied kidney is an ischemia as the ratio of blood flow to the amount of surviving parenchyma must be known to decide this. These considerations are offered as a possible explanation of this case which must otherwise be classified as one of nephrocalcinosis complicated by an independent malignant essential hypertension.

The stimulus to enlargement of the parathyroid glands so regularly accompanying renal insufficiency is not known. The three parathyroids showed an enlargement of approximately 465 per cent by weight, resulting from hyperplasia of the epithelial components. Notwithstanding this, the serum calcium was normal and the serum phosphorus only slightly elevated. From this it may be assumed either that the enlarged parathyroids effectively controlled the concentration of calcium and phosphorus in the blood, or that a high serum phosphorus is not the immediate stimulus to the hyperplasia.

The total weight of the three glands found at autopsy was 399 mg. Pappenheimer and Wilens<sup>6</sup> give 85 to 86 mg. as the mean weight of the three corresponding parathyroid glands in a series of miscellaneous non-nephritics over the age of 10 years. Gilmour and Martin's weights<sup>20</sup> are essentially in accord. The very small amount of thymus IV and thymus III<sup>21</sup> found associated with the left upper and the left lower parathyroid respectively in this case probably did not significantly add to their weights.

The histology of the glands corresponds to that described by Castleman and Mallory as characteristic of secondary hyperplasia. In addition there was another alteration not stressed by them, namely a progressive clearing of the cytoplasm of the chief cells.

The histology of the parathyroid tumor removed at operation differs greatly from that of the normal parathyroid and of the glands found at autopsy. The rim of normal appearing tissue at its periphery leaves little doubt that it represents a true neoplasia rather than hyperplasia. Castleman and Mallory's classification<sup>22</sup> of the pathology of the parathyroid gland would suggest that it belongs in the category of "single chief cell neoplasia." In both the tumor and the hyperplastic glands there is a strong suggestion of transition between cell types.

Our case may be interpreted as one of primary hyperparathyroidism associated with an adenoma of the right lower parathyroid which was removed after extensive calcification of the renal pyramids had occurred. After an interval of 4 years there developed hypertension presumably associated with the arteriolar sclerosis; this served at least in part to precipitate the uremic state in which the patient died. The enlargement of the remaining parathyroids is regarded as secondary to the renal insufficiency.

### SUMMARY

1. A case of hyperparathyroidism is reported associated with an adenoma of the right lower parathyroid, accompanied by calcification of a horseshoe kidney as disclosed by X-ray. Removal of the parathyroid tumor was followed by termination of the hyperparathyroidism. There developed subsequently hypertension, cardiac hypertrophy, renal insufficiency and death in uremia.

2. The important lesions disclosed at autopsy were extensive calcification of the renal pyramids of a horseshoe kidney, widespread arteriolar sclerosis and necrosis, arteriolar nephrosclerosis, and hyperplasia of the three remaining parathyroid glands.

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## DESCRIPTION OF PLATES

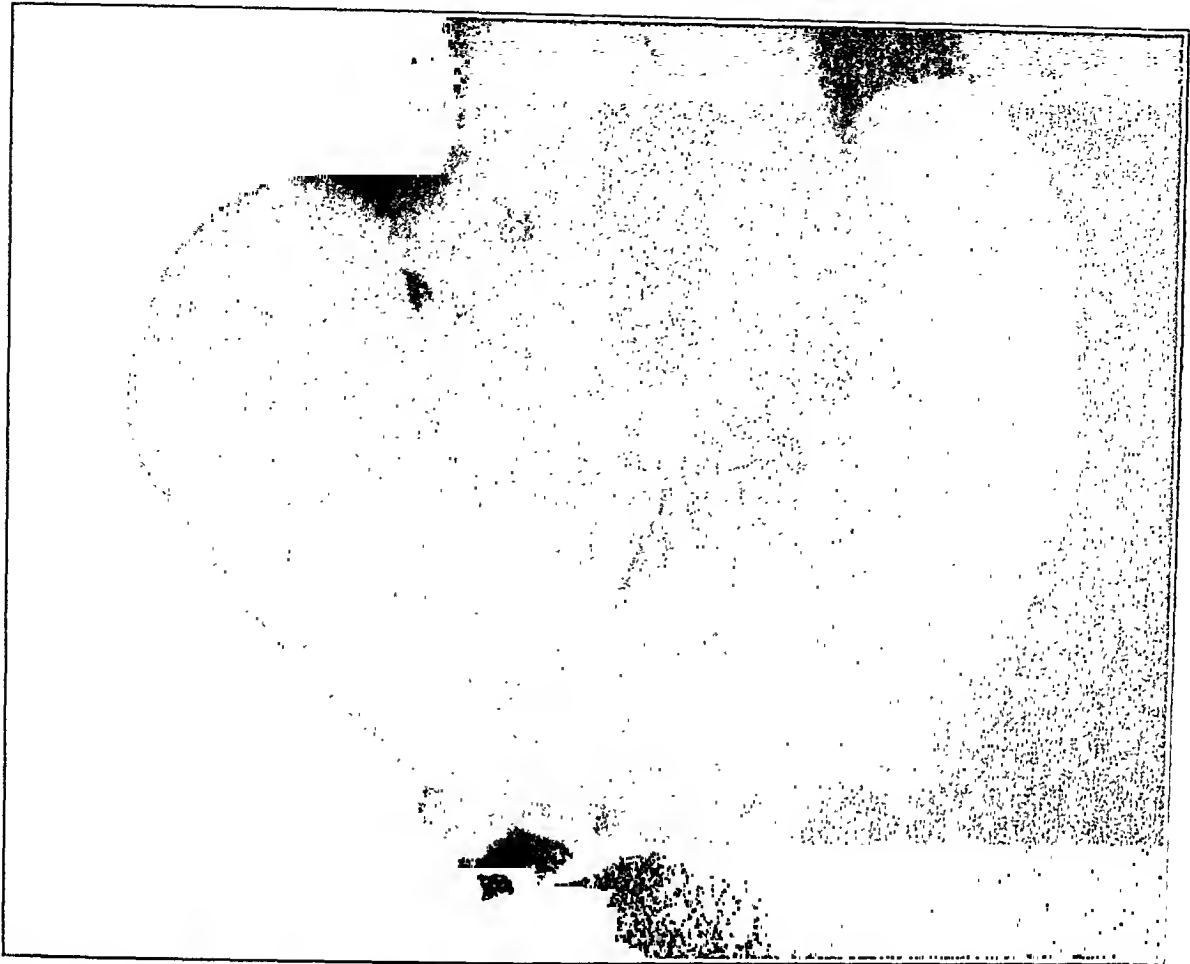
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### PLATE 25

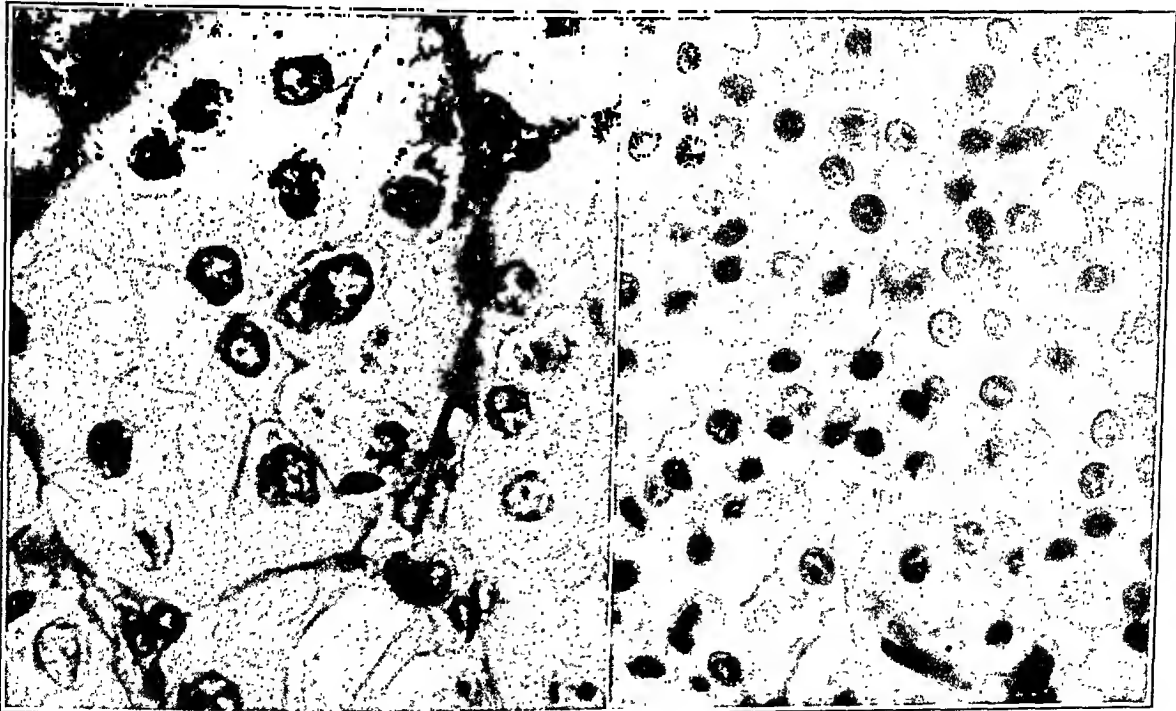
FIG. 1. Roentgenogram of horseshoe kidney removed at autopsy showing calcium deposits in pyramids and relative freedom of cortex and columns of Bertini from calcification. A calcified plaque is demonstrated in the aorta.

FIG. 2. Microphotograph of tumor of right lower parathyroid removed at operation showing large cells grouped in nests. Hematoxylin-eosin stain.  $\times 700$ .

FIG. 3. Left lower parathyroid removed at operation, showing transitional forms between chief cells and cells resembling chief cells but with clear cytoplasm. Hematoxylin-eosin stain.  $\times 700$ .

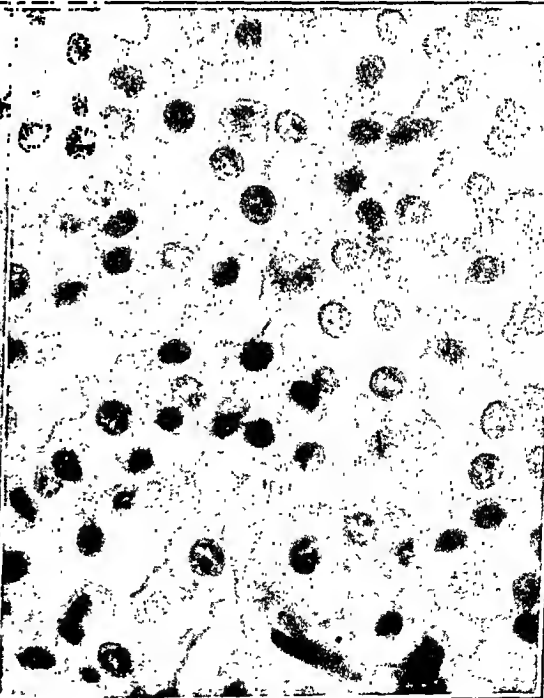


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Johnson



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Primary Hyperparathyroidism

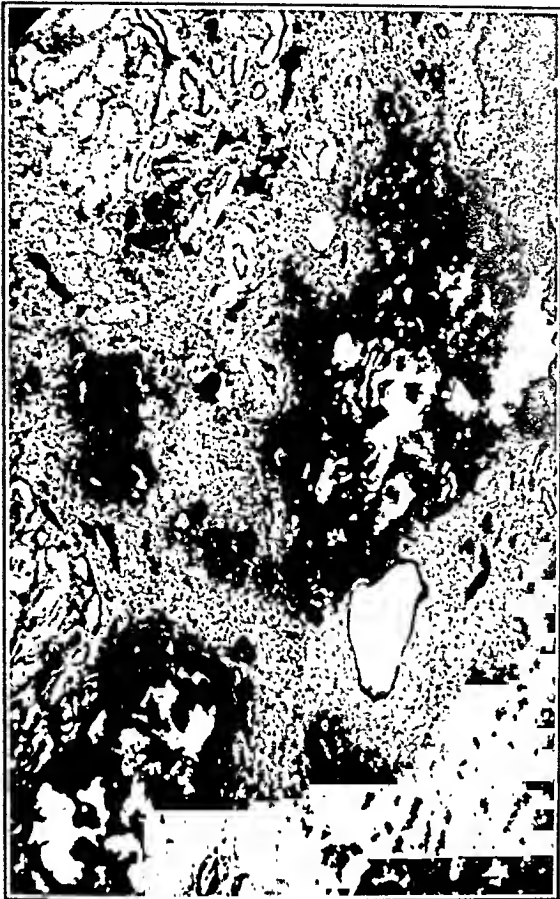
PLATE 26

FIG. 4. Massive deposits of calcium in pyramids of kidney and moderate dilatation of tubules. Hematoxylin-eosin stain.  $\times 60$ .

FIG. 5. Advanced arteriolar nephrosclerosis, plugging of tubules by calcium and deposition of calcium in partially occluded arterioles. Hematoxylin-eosin stain.  $\times 110$ .

FIG. 6. Extensive intimal thickening with hyalinization of a pancreatic arteriole. The lumen is almost completely occluded. Hematoxylin-eosin stain.  $\times 110$ .

FIG. 7. Adrenal arteriole, extensive arteriolar necrosis with swelling and hyalinization of all elements of the wall and complete occlusion of the lumen. Hematoxylin-eosin stain.  $\times 110$ .



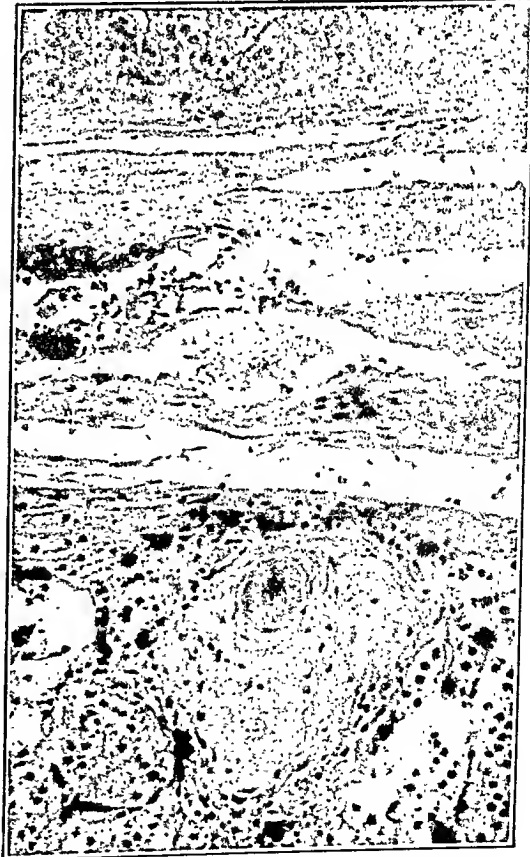
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## TULAREMIC SEPTICEMIA \*

### REPORT OF A CASE

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A case of tularemia with certain characteristics of an associated specific meningitis, which have not been emphasized heretofore, is reported. The primary lesion in the skin, which has been described only once in human beings, was available for histological study 19 days after the initial infection.

### REPORT OF CASE

*Clinical History:* O. E., a white female, 52 years of age, was admitted to the hospital unable to speak and able to swallow only with great difficulty. Fifteen days before admission the patient was bitten on the left shoulder by a tick. Eight days later she complained of soreness in the left chest, associated with a slight cough, and at this time she felt that she had some fever. Two days later she began to have difficulty in swallowing and was unable to speak. The patient at no time complained of headache or vertigo. The past and family history were non-contributory to the present illness.

*Physical Examination:* The patient was well developed, obese, and semi-comatose, unable to answer questions and mildly resistant to examination. The pupils reacted to light and accommodation normally and equally. Examination of the fundus revealed tortuosity and silver wire deformity of the vessels. The neck was rigid. Examination of the chest revealed no pathological findings on auscultation, percussion or palpation. The abdomen was slightly rigid and generally tender. All reflexes were hyperactive. A bilateral positive Kernig sign was elicited. A round ulcerous lesion with a scaly center, resembling herpes, was noted on the left shoulder. A lumbar puncture was performed and 45 cc. of cloudy yellow fluid under considerable pressure were removed.

*Laboratory Examination:* Urinalysis was essentially negative except for acetone and a few cells. Examination of the blood on the day of admission showed: red blood cells, 4,410,000; hemoglobin, 70 per cent; white blood cells, 11,100; polymorphonuclears, 77 per cent; and lymphocytes, 23 per cent.

An agglutination reaction 15 days after the tick bite was 1:160 for tularemia. Blood cultures remained sterile throughout hospitalization. Spinal fluid examination revealed on the day of admission, 1390 white blood cells with 94 per cent lymphocytes, sugar 13 mg., and chlorides 698 mg. Smears remained negative at all times for organisms. The total white blood cell count progressively fell to 110 with 91 per cent lymphocytes.

*Course of Illness:* Four more lumbar punctures were performed, each yield-

\* Received for publication July 8, 1938.

ing cloudy fluid under pressure. Tache cérébrale was noted. Crepitant râles were present over the left lower chest posteriorly, associated with increased voice sounds.

The temperature on admission was 104.8° F. Fever continued throughout the patient's illness, the temperature never going below 102.8°, and it was recorded as 104.8° F. just before death, 4 days after admission to the hospital.

### PATHOLOGICAL FINDINGS

External examination reveals nothing of pathological interest except the left shoulder. On the posterior surface is a round, punched-out ulcer measuring 1.5 cm. in diameter and approximately 0.3 cm. in depth. The edges are somewhat ragged and there is a small amount of encrustation at the base and along the walls. It is surrounded by a purplish discolored area and numerous small vesicles which are situated intra-epithelially.

*Lungs:* The pleura is diffusely covered by fine fibrinous tags and shows numerous, firm, round raised nodules, varying in size from 0.3 to 1.5 cm. in diameter, which are made up of smaller nodules which have in places coalesced. The cut surface reveals diffusely distributed, gray, nodule-like foci in acinous arrangement with yellow necrotic centers. The structures around the hili are negative except for a few enlarged lymph nodes with coalescent areas of caseation.

*Spleen:* The spleen weighs 280 gm. and measures 15 by 12 by 2 cm. The capsule is wrinkled and the organ is soft. The cut surface is dark red and the follicles and trabeculae are barely visible. There are several, large, (0.2 to 0.3 cm.) firm yellow nodules lying within the splenic pulp, which can be scraped away on the knife edge.

*Liver:* The liver weighs 1700 gm. and measures 30 by 23 by 9 cm. There are pin-head-sized and smaller tubercle-like foci diffusely scattered over the cut surface, which in places are definitely situated between the lobules.

*Head:* The scalp and calvarium are negative. The meninges present a milky opacity and show innumerable, pin-head-sized tubercle-like foci diffusely scattered throughout the subarachnoid space over all parts of the brain. There is a slight amount of turbid, yellowish gelatinous exudate in the subarachnoid space, rather diffusely distributed but most prominent at the base of the brain.

## MICROSCOPIC EXAMINATION

Characteristic tularemic nodular areas of necrosis are found in the spleen, liver, lungs, lymph nodes, skin and meninges.

*Lungs:* Sections through various parts of the lungs show two types of lesions.

(1) Numerous nodules with epithelioid cells and central caseation are present in the interstitial tissue. Wherever they encroach upon a vessel they produce complete necrosis of the wall. Intimal proliferation of small vessels in the vicinity of these foci is occasionally encountered.

(2) Areas of caseous bronchopneumonia, some of which are acinous in distribution, are found in all sections. The exudate consists mainly of large mononuclear cells, plasma cells and some fibrin, the latter often clumped. In places this exudate is necrotic. In other areas where the alveolar wall is still intact the vessels are patent but often engorged, and minute hemorrhages frequently occur. The alveolar walls in other areas are necrotic.

In addition to these specific changes there are widely scattered areas of non-specific bronchopneumonia and aspiration pneumonia with recognizable foreign material in the alveolar lumens.

*Brain:* The meninges are edematous. The subarachnoid space shows an exudate composed of polymorphonuclear cells and a variety of round cells, among which large mononuclear cells predominate. These are actively phagocytic, containing small mononuclear cells, nuclei and nuclear fragments, and are frequently foamy in appearance. In some areas the large mononuclear cells accumulate and form rather discrete nodular foci. Fibrinous exudate is seen within and around these foci. Polymorphonuclear cells are rather scanty and epithelioid and giant cells are absent. The foci are irregularly distributed but the majority are found to be independent of vessels, even in the early stage. However, they encroach occasionally upon a large artery producing necrosis of all coats of the wall with proliferation and infiltration of the intima. Thrombi are not encountered. Non-specific round cell infiltration follows the perivascular lymph spaces of some of the smaller vessels into the outer layer of the cortex. This infiltration, however, does not form specific nodules.

Many of the meningeal foci invade the cortical substance, pro-



gressing into the uppermost zone and infiltrating the outer layer of ganglion cells. A Nissl stain reveals that the ganglion cells resist the destructive process for a long time. Although axis cylinders and myelin sheaths have been destroyed and the ganglion cells are compressed within such areas, they reveal normal distribution of tigroid substance and normal nuclei. Their processes seem, however, often to be unduly swollen.

Sections taken from the pons, brain stem, Ammon's horn and various portions of the cortex appear to be normal.

*Skin:* A section from the area of the tick bite shows a deep ulcer, the base of which is within the level of the corium although close to the subcutaneous fat tissue. Its superficial layer is composed of débris containing numerous bacteria, mostly staphylococci, either isolated or in clumps. Beneath this is a layer of round cell infiltration consisting mainly of polymorphonuclear cells. The vessels beneath the ulcer are considerably engorged and there are numerous areas of hemorrhage scattered throughout the corium.

Another section taken from an area close to the tick bite shows the formation of a vesicle. The epidermis covering an area of dense infiltration is somewhat thickened. There is slight acanthosis, but most of the thickening seems to be due to a degenerative process in the prickle cell layer. The stratum corneum is lifted and forms the top of the vesicle; the base is level with the stratum lucidum.

A few, apparently early specific lesions are found just beneath the epithelial surface. Discrete areas of necrotic connective tissue are infiltrated, particularly at the periphery, with round cells, mostly of the lymphocytic type, and with a few polymorphonuclears. The infiltrating cells undergo rapid necrosis and a dense aggregation of nuclear fragments is seen between the necrotic masses of connective tissue. These areas of nodular necrosis border apparently normal connective tissue. There is no peripheral zone of monocytic or epithelioid cells. At one point this focus encroaches upon a small vessel, the wall of which is completely necrotic. There is some hemorrhage into the necrotic area.

For some distance from the base of the ulcer, separated from the surface by intact connective tissue, a few nodular areas of necrosis are seen resembling very closely the nodules found in

other organs. At the periphery of these necrotic areas large mononuclear and epithelioid-like cells are present together with a rather dense infiltration of round cells and a few polymorphonuclears. One of these nodules is situated at the root of a hair follicle destroying the squamous epithelial cell layer. It is associated with an accumulation of polymorphonuclear cells within the epithelial lining of the follicle resulting in necrosis of the hair shaft itself. The vessels of the papillae between the nodule and the epidermis are considerably engorged. Rather dense infiltration with lymphocytes is seen in perivascular and perifollicular spaces and around the sweat glands throughout the section. Some arterioles leading to specific foci show complete necrosis of the media, but the adventitia and intima are well preserved. The walls of other vessels are completely invaded by granulation tissue with destruction of the intima and early fibrinous thrombosis.

#### COMMENT

Both in gross and microscopically, the case presents all the characteristics of tularemia. However, certain observations warrant a discussion.

1. *The Meningitis:* Tularemic meningitis has been reported three times (Bryant and Hirsch,<sup>1</sup> Pund and Hatcher,<sup>2</sup> and Haizlip and O'Neil<sup>3</sup>). It has been stressed that in gross the lesions resemble tuberculous meningitis, except for the fact that in tularemia the base of the brain is involved to a lesser degree than the cortex and the lateral aspects of the hemispheres. Histologically, tuberculous and tularemic meningitis are almost identical. In each the nodular granulation tissue tends toward rapid necrosis, giant cells are scanty, and epithelioid cells poorly differentiated. There is a tendency in each to diffuse spread of a subarachnoid exudate which is predominantly of a mononuclear cell type and not specific in nature.

The distribution of nodules in our case of tularemia, however, differs strikingly from that seen in tuberculosis. While the nodules in the latter condition have a distinct tendency to follow the vessels, particularly into the depths of the sulci, there is an irregular distribution in our case independent of the course of the vessels with the site of the nodules preferably at the vertex of the gyri.

This difference is likewise noticeable in histological sections.

In tuberculous meningitis the lesion usually begins in the adventitia of the vessels and it is characteristic to find the epithelioid tubercles or foci of caseation in later stages predominantly in perivascular arrangement. Involvement of the media and intima is common, frequently resulting in occlusion of arteries by specific or non-specific endarteritis. Although the tuberculous process may occasionally encroach directly upon the brain tissue, there is, in general, little tendency to destructive invasion through the glial membrane.

In our case of tularemic meningitis the relations are almost reversed. The lesions begin some distance from the vessels where large mononuclear cells accumulate and form discrete nodular foci. The fully developed nodules of caseation in later stages seem almost to avoid the vessels, most of which are intact. The caseating granulation tissue only occasionally encroaches upon the wall of a vessel. The tularemic nodules, furthermore, show a distinct tendency frequently to invade the adjacent cortical tissue. In their description of tularemic meningitis, Bryant and Hirsch mention that the lesions were distributed chiefly, although not invariably, along the vessels. With the exception of this report we could not find specific statements in the descriptions of other cases of tularemic meningitis with reference to the relation of nodules to vessels. Their irregular distribution and their independence of vessels in our case are most striking and if this tendency is as salient as we noticed it to be, it may be of value in regard to the differential diagnosis from tuberculous meningitis.

2. *The Primary Lesion:* The primary skin lesion 15 days after infection has been described by Goodpasture and House.<sup>4</sup> Woolley<sup>5</sup> briefly reports the histological changes in primary skin lesions of experimental tularemia in laboratory animals. Goodpasture emphasizes the fact that the necrosis extends into the surrounding tissue particularly about the arteries which, however, are left relatively unimpaired.

In our case the intradermal spread occurred in a rather irregular fashion within the lymph channels independent of vessels. The cellular infiltration, which in areas showed a tendency to necrosis, was found in periglandular, perivascular and perineural lymph spaces. Nodules of mononuclear cells with caseation were also found isolated, in no relation to any one of the above men-

tioned structures of the skin. They encroached, however, not infrequently upon smaller vessels resulting in necrosis of a segment or the entire circumference of their walls with subsequent subintimal infiltration and proliferation.

The primary ulcerating lesion spread into the surrounding skin within a distance of several centimeters, causing numerous small blisters. The latter histologically resemble impetigo. The vesicles are situated within the epidermis proper, the horny layer lifted to form its roof. Hair follicles are involved in a suppurative process. It seems reasonable to assume that this vesicular dermatitis surrounding the primary lesion is due to cutaneous autoinoculation. It must, however, be emphasized that specific nodules were found independent of hair follicles. Our findings do not permit a definite conclusion with reference to the mechanism of intradermal or epidermal spread. It is most probable, however, that it follows the lymph channels.

3. *Tularemic Septicemia*: The involvement of rather numerous and engorged vessels in the specific inflammatory process of the skin would explain the mechanism of further hematogenous spread had it not been that Goodpasture's findings shed doubt upon this interpretation. The intracutaneous vessels in his case are described as relatively unimpaired.

It is of interest to note that most cases of tularemic septicemia lack the embolic type of metastases in the organs of the greater circulatory system. The case of Permar and Maclachlan <sup>6</sup> possibly represents an exception in that an anemic infarction occurred in the spleen which was believed to be due to thrombosis of a pulmonary vein. It is, however, uncertain whether this infarction was specific in nature or not. Metastases most frequently are confined to the lungs, lymph nodes, and organs with reticuloendothelial cells (mainly liver and spleen). Foci in other organs are rare exceptions (Pund and Hatcher <sup>2</sup>). Lymph nodes are often preferably involved. This distribution indicates that no special focus of development, such as a thrombophlebitis, is interposed between the focus of entrance and the spread of infection. In this respect tularemic septicemia differs from tuberculous septicemia (miliary tuberculosis) in which the focus of development can be detected in the great majority of cases as caseous thrombophlebitis of pulmonary veins or caseation of the thoracic duct.

Tularemic septicemia, furthermore, is distinguished from miliary tuberculosis in that it shows a tendency to become localized in lymph nodes, particularly those draining organs with tularemic foci of infection. It is characteristic to find caseation in gross in the hilar lymph nodes of the lung and liver if tularemic foci are localized in these organs.

4. *Involvement of Vessels*: It has been stated that endothelial and subendothelial proliferation frequently occur, resulting in narrowing of the arterial lumens, thereby causing the caseating necrosis of the tissue (Permar and Weil,<sup>7</sup> and Gundry and Warner<sup>8</sup>).

Although vascular changes with narrowing of the lumen were observed in our case, they were scanty, scattered and mostly confined to the lesions in the lung. The process of caseation, however, appeared to be independent of the vascular lesions. Extensive areas of necrosis were found in the lung in which the alveolar walls contained patent capillaries engorged with blood. In fact, hemorrhages into such foci from capillaries supplying these areas were fairly frequent. This proves that necrosis occurs while the blood supply is still intact. We conclude that the coagulation necrosis in tularemia does not depend on vascular changes and is not ischemic in origin.

#### SUMMARY

1. A case of tularemic septicemia is presented with special reference to the primary skin lesion and the meningeal involvement.

2. The difference in distribution of lesions in tularemia and miliary tuberculosis is discussed and the following conclusions reached: (1) Tularemic meningitis is characterized by relative independence of nodular foci of the course of vessels. (2) Lymph nodes draining organs involved in tularemia show frequent caseation. (3) The distribution of nodular foci of infection in tularemic septicemia indicates that no intermediate focus of development is interposed between the point of entrance and the metastasis.

3. The process of caseation is not due to lack of blood supply since areas of caseation containing patent and engorged capillaries are demonstrated.

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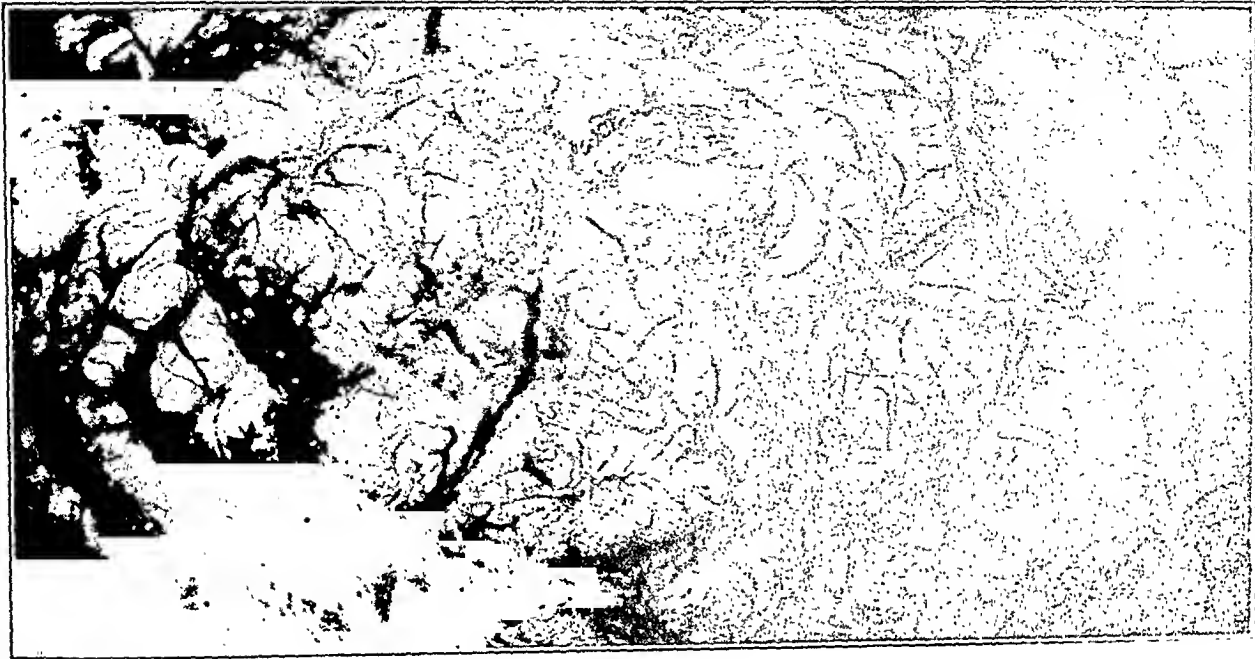
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## DESCRIPTION OF PLATE

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### PLATE 27

- FIG. 1. Lateral view of brain. The tularemic nodules are rather large and appear relatively independent of the course of the vessels.
- FIG. 2. Section of meninges showing a tularemic nodule fully developed and independent of the blood vessels. Even the blood vessel close to the large nodule shows a sharp line of demarcation.
- FIG. 3. Blood vessel immediately beneath the primary lesion of the skin where the granulation tissue destroyed a segment of the wall, resulting in rupture of the endothelium.



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2



3





# CONGENITAL RHABDOMYOMA OF THE HEART \*

## REPORT OF A CASE

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(Third Division, New York University), Bellevue Hospital,  
New York City)*

Von Recklinghausen<sup>1</sup> in 1862 was the first to report a case of congenital rhabdomyoma of the heart. Since then reports of 49 other cases have appeared in the medical literature. In America, cases have been recorded by Knox and Schorer (1906),<sup>2</sup> Wolbach (1907),<sup>3</sup> Farber (1931),<sup>4</sup> and Ill and Gray (1934).<sup>5</sup> The case here reported makes the 5th in American and the 51st in the general medical literature.

## REPORT OF CASE

*Clinical History:* The mother of the infant to be discussed was a white woman, 24 years of age. The past history was irrelevant. The Wassermann reaction was negative. She had a normal spontaneous delivery of a child, now living and well, on May 9, 1934. A spontaneous abortion at 3 months occurred in August, 1934.

Later, the birth of a male infant, weighing 4215 gm., occurred on April 29, 1937, on the obstetrical service of Bellevue Hospital. At birth the child was cyanotic. Respiration was artificially established 3 minutes after birth, but the cyanosis continued. Normal temperature could be maintained only through the application of external heat. Physical examination of the baby was negative except for cyanosis and a short systolic murmur which was heard over the pulmonary cardiac area. The baby lived only 3 hours.

## POSTMORTEM EXAMINATION

The body is that of a well developed white male infant weighing 4215 gm. and measuring 53.3 cm. in length. The skin appears normal except for a moderate amount of postmortem lividity in the dependent portions of the trunk.

With the exception of the heart and brain, other organs show nothing of importance.

*Heart:* The heart is enlarged to twice its normal size and weighs 49 gm. The epicardium appears normal. On the left border of the

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heart, close to the apex, there is a raised nodule 1 cm. in diameter, which on section is seen to be composed of a circumscribed area of soft brown tissue arranged in whorls.

The pulmonary artery is dilated and measures 1 cm. in diameter at its base. The ductus arteriosus is dilated also, measuring 8 mm. in diameter, but the ascending aorta measures only 5 mm. It would appear that the main flow of blood was through the ductus arteriosus rather than through the ascending aorta. The descending aorta appears normal.

On sectioning the heart the right auricle and tricuspid valves appear to be normal. A rudimentary interauricular septum imperfectly separates a rudimentary left auricle, measuring 1.5 cm. in thickness, from the right auricle. The foramen ovale cannot be identified. The left auricle through the atretic mitral valves communicates with a small cavity 1.5 cm. in diameter representing the left ventricle. The aortic valves are bicuspid. The right ventricle is lined with glistening endocardium. The papillary muscles are hypertrophied and the base of one of the muscles within the right ventricle has been invaded by a tumor measuring 1.5 cm. in diameter. The left ventricle is lined with glistening gray endocardium 2 mm. in thickness. Adjoining the endocardial lining is a layer of dense gray tissue 2 mm. thick. The wall of the right ventricle is 1 cm. thick. The wall of the myocardium representing the interventricular septum measures 3 cm. and separates the rudimentary left ventricle from the large right ventricle.

On section of the interventricular septum two distinct tumor masses are seen. One measures 2.5 by 1.5 cm. It is well encapsulated, gray in color, and extends into the base of the papillary muscle as mentioned above. The cut surface of the tumor presents a grayish, granular dimpled appearance with faint, peripheral radial striations adjacent to the capsule. The capsule is 1 to 2 mm. thick and is smooth, gray and glistening.

The second tumor, 2 by 2.5 cm., is adjacent to the one described above and extends through the remainder of the interventricular septum and the anterior wall of the right ventricle. The tumor is not encapsulated but is well defined from the surrounding myocardium. It is composed of smooth, firm, glistening gray tissue somewhat fibrous in appearance. Scattered through the myocardium at the apex of the heart are a number of well circum-

scribed, light brownish islands of tissue of the same consistence as the myocardium.

*Brain:* On opening the cranium a moderate amount of sub-arachnoid edema is found and the cerebral vessels appear moderately congested. On section of the brain the subependymal tissue of the lateral ventricles is markedly thickened. On the floor of the left lateral ventricle extending posteriorly through the left occipital lobe is a tumor 6 by 2 cm. composed of well circumscribed reddish brown tissue. The tumor is firmer in consistence than the surrounding brain tissue. There is a moderate degree of internal hydrocephalus.

#### MICROSCOPIC EXAMINATION

*Heart:* A hematoxylin and eosin stain of tissue taken from the interventricular septum shows a tumor with a thin connective tissue capsule which varies in thickness, and in a few areas the tumor lies directly superimposed on myocardial tissue. A number of fair sized blood vessels with thin walls are seen within the capsule. Short connective tissue septums dip into the tumor, carrying small and medium sized blood vessels. A few small or medium sized arteries are also found within the tumor.

The myocardium immediately overlying the capsule is composed of delicate and elongated muscle fibers with prominent nuclei and cross striations and separated by slender connective tissue fibers containing a few fresh red blood cells and leukocytes.

The tumor is characterized by many, usually rounded vacuolated spaces which vary in size (Figs. 1 and 3). The tissue between them is composed of large polygonal branching cells with a finely granular and eosinophilic cytoplasm (Fig. 3). A fine fibrillar network is associated with these cells. Three or four cytoplasmic processes spring from the surface of each cell body and many of the cells contain round, deeply staining nuclei. In some instances the cells appear to lie within the vacuoles, the cytoplasmic processes spreading out to subdivide the space (Fig 2).

With high magnification the vacuolated spaces are seen to be lined by a thin rim of amorphous tissue. Often the lining of these spaces appears to be derived partially from the cytoplasmic process of an adjacent large granular cell (Fig. 3). The spaces appear to be extracellular and the large polygonal branching cells

lie between them. The majority of the spaces are clear but a few, however, contain several small round eosinophilic cells with bilobed dark nuclei resembling polymorphonuclear leukocytes (Fig. 1).

The cytoplasm of the cells contains many fine eosinophilic granules arranged to produce cross striations (Figs. 1 and 2). Under oil immersion the cross striations become even more evident and are seen to extend from the main body of the cell into the branching processes, which number two to six per cell. Most of the cells contain a single, large round nucleus with prominent nucleoli and chromatin granules. A few of the cells contain two nuclei.

Sections of the apparently normal myocardium disclose small foci of tumor tissue similar to that described above. A section of the second tumor mass shows tissue resembling a fibromyxoma, representing, probably, degenerative changes.

*Brain:* On microscopic examination the tumor in the occipital lobe is found to be a spongioblastoma with unipolar, bipolar and multipolar cells. Between the spongioblasts are large numbers of small, oval and round nuclei which cannot be identified. Every portion of the brain examined shows some deviation from normal. The main changes are noted in the occipital and frontal poles on both sides. Here the cortical architecture is distorted. Many abnormal nerve cells are present as well as enormous astrocytes and numerous undifferentiated cells. No ependymal cells are found lining the ventricles, but replacing them is a dense layer of glia fibers. No myelin formation is noted in any of the sections.

*Other Organs:* The lungs are congested and show focal hemorrhages with foci of aspirated amniotic fluid. Icterus is present in the liver. The kidneys are congested. The spleen shows acute splenitis.

*Pathological Diagnoses:* Primary congenital rhabdomyoma of the heart with multiple tumors; congenital malformation of the heart with atrial septal defect, hypoplasia of the aorta, mitral stenosis, dilatation of the pulmonary artery and ductus arteriosus, bicuspid aortic valve, and rudimentary left auricle and left ventricle; and tuberous sclerosis of the brain with cerebral tumor.

## DISCUSSION

In Table I are summarized the cases reported to date of congenital rhabdomyoma of the heart.

Of the 51 cases the tumor was classified as single in 8, multiple in 36, and diffuse in 3. Two cases showed two nodules, 1 case showed no tumor in gross, and 1 other case is unquestionably a rhabdomyoma from the photographs. Tuberous sclerosis of the brain was found associated with the tumor of the heart in 29 cases. In 4 cases tuberous sclerosis was not found and in 18 cases no mention of this lesion is made. Other congenital lesions were found also in association with the rhabdomyoma of the heart. Thus, in 24 cases tumors or cysts of the kidneys were reported. Cutaneous tumors were seen in 3, an enlarged liver or spleen in 3, and harelip or cleft palate in 1 case. Thirty-one cases had some associated lesion in organs other than the brain or heart. The age distribution of this tumor was found to be as follows: newborn, 10; under 1 year, 15; 1 to 3 years, 8; 3 to 15 years, 11; and over 15 years, 5. The age was not given in 2 cases.

As evidence of the congenital nature of rhabdomyoma of the heart, its occurrence in the newborn and its association with congenital anomalies have been noted. Cleft palate, harelip, cystic kidneys, tuberous sclerosis, multiple gliomas of the brain, hypernephroma, sebaceous gland adenomas, embryonic rests in the kidney, and embryonic malformation of the pancreas are among the more important congenital anomalies found associated with rhabdomyoma of the heart. Neuropathologists have noted that congenital anomalies are often associated with tuberous sclerosis of the brain.

Steinbiss<sup>6</sup> believes that the congenital rhabdomyomas of the heart cannot be regarded as true tumors. They do not show any evidence of proliferative activity, and degenerative changes such as fibrosis and calcification have been noted in older lesions. In the case here reported, a small portion of the tumor showed fibrotic degenerative changes. Malignant transformation of the tumor has not been recorded and probably does not occur. Farber<sup>4</sup> gives an excellent review of this controversial question. He believes that the evidence is against a neoplastic nature of the tumor. Rehder,<sup>7</sup> and Schmincke,<sup>8</sup> as well as Steinbiss believe that

TABLE I  
Data on Cases of Congenital Rhabdomyoma of the Heart Reported in the Literature to Date  
(Enlarged after Farber)

Number	Author	Year	Age	Sex	Histological findings		
					Heart	Brain	Other organs
1	Von Recklinghausen <sup>1</sup>	1862	Newborn	—	Multiple nodules in both ventricular walls	Tuberous sclerosis	Cutaneous tumors
2	Virchow <sup>21</sup>	1864	Newborn	—	Multiple nodules in both ventricular walls	—	Hepatomegaly, multiple skin tumors
3	Hlava <sup>22</sup>	1887	14 days	—	Single tumor in left ventricle	Not examined	—
4	Kolisko <sup>18</sup>	1887	2 mos.	—	Multiple small nodules	—	—
5	Cesaris-Demel <sup>20</sup>	1895	3 yrs.	—	Multiple tumors in both ventricles and septum	Tuberous sclerosis	Small nodular renal tumors, embryonic renal tissue without glomeruli
6	Seiffert <sup>17</sup>	1900	20 mos.	M	Multiple tumors in apex, myocardium and septum	—	Cystic kidney
7		1900	7 mos.	—	Multiple small myocardial tumors	—	—
8	Rothe <sup>23</sup>	1901	—	—	Multiple tumors	Tuberous sclerosis	Multiple breast tumors
9	Ponfick <sup>10</sup>	1901	7 mos.	M	Multiple nodules in both ventricles	Tuberous sclerosis	—
10		1901	3 yrs.	F	Multiple nodules in both ventricles	Tuberous sclerosis	—
11	Bonome <sup>19</sup>	1902	1½ yrs.	—	Multiple tumors	Tuberous sclerosis	—

12	Riedmatten <sup>21</sup>	1904	1½ yrs.	—	Multiple tumors	Tuberous sclerosis	—
13	Knox and Schorer <sup>2</sup>	1906	7 mos.	—	Multiple tumors, one large pedunculated tumor in left ventricle	—	—
14	Wolbach <sup>3</sup>	1907	10 mos.	F	Single tumor in right ventricle	Negative	Neuroglioma of spinal meninges
15	Abricsoff <sup>12</sup>	1909	3¼ yrs.	—	Multiple tumors in both ventricular walls	Tuberous sclerosis	—
16	Ehrnrooth <sup>25</sup>	1911	7 mos.	—	Single tumor in left ventricle	Grossly negative	Negative
17	Bundschuh <sup>23</sup>	1912	2 yrs.	F	Multiple nodules	Tuberous sclerosis	Tumors of kidneys, glioma of dura, adenoma sebaceum
18	Jonas <sup>27</sup>	1912	6 mos.	M	Multiple nodules in both ventricular walls	Tuberous sclerosis	Congenital malformation of kidney, hare-lip, cleft palate
19	Kawamura <sup>9</sup>	1913	4 yrs.	F	Multiple nodules in both ventricles	—	Renal tumors, congenital anomalies in pancreas, esophagus and rectum
20	Schulgin <sup>10</sup>	1913	6 days	—	Multiple tumors in both ventricles	Tuberous sclerosis	Kidney tumors
21			6 yrs.	—	Multiple tumors in both ventricles	Tuberous sclerosis	Kidney tumors
22	Rehder <sup>7</sup>	1914	Newborn	—	Multiple nodules	—	Grossly negative
23	Mönckeberg <sup>11</sup>	1914	14 mos.	—	Multiple nodules	Tuberous sclerosis?	Absent right kidney and ureter
24	Ribbert <sup>23</sup>	1915	1 yr.	—	Multiple nodules	Tuberous sclerosis	Cysts of kidneys
25	Hisinger-Jägerskiöld <sup>20</sup>	1916	7½ mos.	—	Single nodule at apex	—	Tumors of kidneys
26	Amersbach and Handorn <sup>25</sup>	1921	7 days	M	Single nodule	Negative	Negative



TABLE I (Continued)

Number	Author	Year	Age	Sex	Histological findings			Other organs
					Heart	Brain		
27	Kaufmann <sup>30</sup>	1922	3 yrs.	-	Multiple nodules	Tuberous sclerosis		Kidney tumors
28		1922	7 yrs.	-	Multiple nodules	Tuberous sclerosis		Kidney tumors
29	Mittasch <sup>31</sup>	1922	4 mos.	-	Multiple nodules	Tuberous sclerosis		Kidney tumors
30		1922	14 yrs.	M	Multiple nodules	Tuberous sclerosis		Kidney tumors, angiomyolipoma of liver
31		1922	31 yrs.	M	Multiple nodules	Tuberous sclerosis		Congenital tumor of lungs
32	Schmincke <sup>8</sup>	1922	Newborn	-	Diffuse involvement of myocardium			Fibroepithelioma of skin, kidney tumors?
33	Steinbiss <sup>9</sup>	1923	5 yrs.	M	Multiple nodules in both ventricles	Tuberous sclerosis		Kidney tumors
34		1923	8 yrs.	M	Multiple nodules in both ventricles	Tuberous sclerosis		Kidney tumors, adenoma sebaceum
35		1923	10 yrs.	M	Multiple nodules in both ventricles	Tuberous sclerosis		Kidney tumors and cysts
36		1923	16 yrs.	M	Nodule in septum and cicatrix	Tuberous sclerosis		Kidney tumors
37		1923	21 yrs.	F	Two small nodules in right auricle	Tuberous sclerosis		Kidney tumors
38		1923	35 yrs.	-	Single nodule at apex of left ventricle	Tuberous sclerosis		Kidney tumors
39	Omodei-Zorini <sup>32</sup>	1923	2½ yrs.	-	Single tumor in right ventricle			

40	Uehlinger <sup>13</sup>	1925	20 yrs.	M	Multiple nodules in left myocardium	Not mentioned	—
41	Berger and Vallée <sup>14</sup>	1930	2 yrs.	—	Multiple nodules	Not examined	Cysts of kidneys
42	Farber <sup>4</sup>	1931	6 mos.	F	Multiple nodules in both ventricles	Tuberous sclerosis	Cysts of kidneys
43	Reitano and Nucciotti <sup>33</sup>	1933	1 day	—	Multiple, one large nodule almost replacing the entire heart	Not mentioned	—
44	Ill and Gray <sup>5</sup>	1934	48 hrs.	M	Multiple nodules	Not examined	Enlarged liver and spleen
45	Mitani <sup>20</sup>	1934	Newborn	M	Single tumor in interventricular septum and muscle of ventricles and auricles	Tuberous sclerosis	Cysts of kidney
46	Wegman and Egbert <sup>31</sup>	1935	10 mos.	F	Multiple nodules	Negative	Cysts of kidneys
47	Heuper <sup>35</sup>	1935	7 mos.	M	None grossly, microscopic foci of tumor tissue	Tuberous sclerosis	Cysts of kidneys, multiple spongioblastomas of basal ganglia, enlarged liver
48	Pauli <sup>37</sup>	1936	4 mos.	M	Diffuse tumor	Not mentioned	Enlarged liver, hydrocele
49		1936	6½ mos.	M	Diffuse tumor	Not mentioned	Not mentioned
50	Tamura <sup>38</sup>	1936	—	—	From photographs is unquestionably a case of rhabdomyoma	—	—
51	Labate	1939	3 hrs.	M	Multiple tumors in right ventricle and interventricular septum	Tuberous sclerosis with spongioblastoma	Congenital anomaly of heart

the tumor is a malformation and not a true tumor. The form of the embryonal cells composing the tumor is retained but the size is greatly increased. There is retardation of development but hypertrophy of the individual elements.

On the other hand, Wolbach<sup>3</sup> with the aid of the phosphotungstic acid hematoxylin stain demonstrated beginning muscle fibril formation and so considered the rhabdomyoma a true neoplasm. He demonstrated that the walls of the vacuolated spaces contain striated fibrils, analogous to the fibrils of normal heart muscle. These striations were observed in studying our case. Many observers have recalled the analogy between the tumor cells and embryonic heart muscle.

Knox and Schorer,<sup>2</sup> Kawamura,<sup>9</sup> Schulgin,<sup>10</sup> Mönckeberg,<sup>11</sup> Abricossoff,<sup>12</sup> Uehlinger,<sup>13</sup> and Berger and Vallée<sup>14</sup> believed that the cells of the tumor arise from Purkinje fibers.

Steinbiss claimed that the tumors were found often where the conduction system could not be demonstrated. Amersbach and Handorn<sup>15</sup> also believed that no connection existed between the tumor and the conduction system. Bonome<sup>16</sup> believed that rhabdomyomas develop from embryonic muscle fibers which become isolated through a connective tissue overgrowth replacing adjacent degenerated muscle fibers.

Wolbach, with Mallory's aniline blue connective tissue stain demonstrated that the sarcous elements stain red and the delicate striations between the sarcous elements (membrane of Krause) blue. In tumor tissue stained in this way the delicate fibrils connecting the fuchsin stained bodies are blue. With the phosphotungstic acid hematoxylin stain they are a reddish brown. These facts justify the belief that the granules of the tumor cells are primitive sarcous elements. The orderly arranged dots, most prominent in the tumor cells and the cell processes, are primitive sarcous elements. The fibrillary material taking the blue of the connective tissue stain is to be regarded as an element similar to that of Krause's membrane in normal muscle.

Von Recklinghausen,<sup>1</sup> Seiffert,<sup>17</sup> Kolisko,<sup>18</sup> Ponfick,<sup>19</sup> and Bonome<sup>16</sup> have noted the similarity of the tumor cells to embryonic heart muscle cells.

Von Recklinghausen considered the vacuolated spaces to be lymph or blood spaces, or muscle tubes of pathological origin.

Cesaris-Demel<sup>20</sup> was the first to call attention to the many processed cells lying within the spaces and called them "spider cells." He regarded the spaces as intercellular and similar to those found between anastomosing cells in embryonal hearts. Seiffert considered the spaces to be intracellular and also called attention to the spider-like cells. He compared these cells with the spaces to huge embryonic cells. Virchow<sup>21</sup> was not certain whether the spaces were lymphatic cavities or clear serous cavities. Hlava<sup>22</sup> believed them to be intracellular artifacts produced by alcohol during fixation. Ponfick, Knox and Schorer and Wolbach considered them to be intracellular.

The presence of glycogen in the vacuolated spaces has been a source of dispute as it is dissolved by solutions used in the ordinary methods of preparing sections. Seiffert was convinced of its presence but could not prove it. Rehder and Mönckeberg demonstrated glycogen in the tumor cells. Farber also demonstrated glycogen in the vacuolated spaces in the tumor in his case and states that others also have found this to be true. We were unable to demonstrate any in the vacuolated spaces of the tumor in our case.

Twenty-nine cases (57 per cent) of congenital rhabdomyoma of the heart were associated with tuberous sclerosis of the brain. Ponfick (1901) was the first to call attention to the associated cerebral sclerosis. He believed that the tumor of the heart and the sclerosis of the brain were congenital. Bonome felt that the association of rhabdomyoma of the heart with cerebral sclerosis was dependent upon the same conditions in intrauterine life, *i.e.* disturbances of nutrition with resulting vascular lesions. Steinbiss also noted the coexistence of rhabdomyoma of the heart with cerebral tuberous sclerosis. The latter may result in imbecility and epilepsy and accounts for the frequent occurrence of the tumor in inmates of hospitals for the insane. The cases reported by Steinbiss ranged in age from 5 to 35 years and all occurred in the insane.

#### SUMMARY

1. The 51st case of congenital rhabdomyoma of the heart has been presented. The tumor occurred in a male infant who lived 3 hours after birth. Multiple tumors were found in the right

ventricle and interventricular septum. The tumor was characterized by many, usually rounded vacuolated spaces. The tissue between them was composed of large polygonal branching cells with a finely granular and eosinophilic cytoplasm. Congenital developmental defects of the heart as well as tuberous sclerosis of the brain with spongioblastoma were found in association with the heart tumor. As evidence of the congenital nature of rhabdomyoma of the heart, its occurrence in the newborn and its association with congenital anomalies have been noted. The true nature of the tumor is not known.

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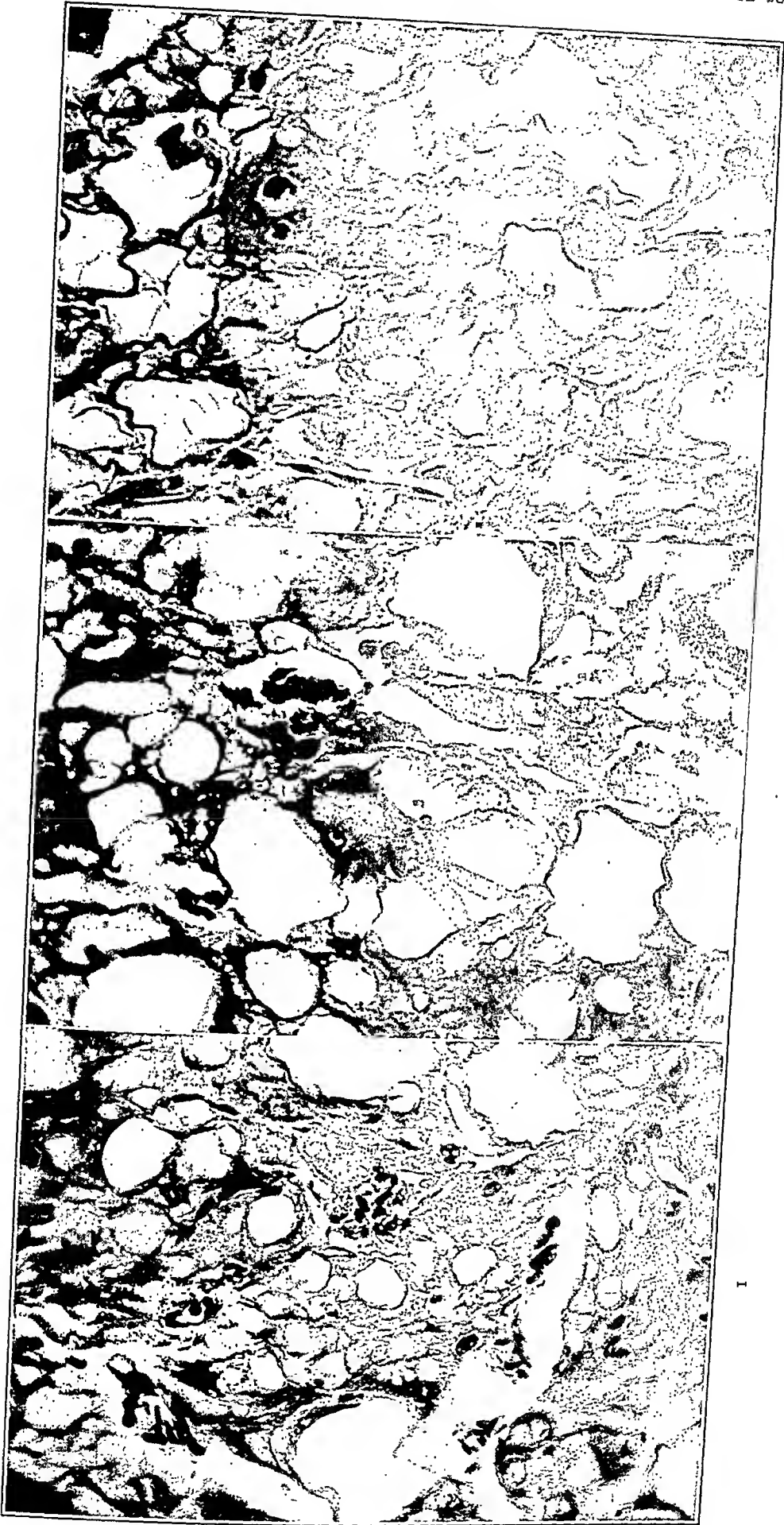
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#### DESCRIPTION OF PLATE

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##### PLATE 28

- FIG. 1. Section from tumor showing the granules arranged to produce cross striations. Note the large number of vacuoles. Hematoxylin-eosin stain.
- FIG. 2. Note the giant spider-like cell, the cytoplasm of which contains granular cross striations, in the vacuolated space. Foot's modification of the Masson trichrome stain.
- FIG. 3. Large intracellular cells are seen occupying the vacuolated spaces. This is a characteristic feature of the tumor. Hematoxylin-eosin stain.



3

I





# A METHOD FOR THE IMPREGNATION OF PERIVASCULAR NERVES ON INTRACEREBRAL BLOOD VESSELS \*

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Study of the perivascular nerve supply of intracerebral blood vessels has always offered many obstacles. Although a wealth of silver impregnation methods is available, they all fall short of demonstrating adequately perivascular nerves on intracerebral blood vessels. In a quantitative study of the richness of the perivascular nerve supply to intracerebral blood vessels the Penfield <sup>1</sup> method was at first used. It was found, however, that although in many instances a clear picture was given, many specimens were lost from over-precipitation of the stain. In the search for a more consistent method the use of activated protargol was attempted. The method as originally described by Bodian <sup>2</sup> for other nerve structures was found to impregnate fibers on intracerebral blood vessels in tissue from animals freshly injected, but only an occasional fiber was impregnated on old, fixed human material. The following modification of the fixative used for fresh animal tissues, and treatment for old, formalin-fixed human material yielded excellent results.

The routine method is as follows:

## A. Human Material Previously Fixed in 10 per cent Formalin:

1. Dissect out the vessels under the dissecting microscope.
2. Wash vessels in distilled water for 12 hours.
3. Place for 4 hours in a solution of alcohol, acetic acid and formalin made up as follows:

Alcohol, 95 per cent . . . . .	90 cc.
Acetic acid (glacial) . . . . .	5 cc.
Formalin . . . . .	5 cc.

4. Wash for 2 hours in distilled water.
5. Place in 1-2 per cent aqueous solution of protargol containing 2 gm. of metallic copper (Merck) per 20 cc. of solution. Leave

\* Received for publication August 1, 1938.

in this solution for 6-24 hours at 35° C., depending on the intensity of impregnation desired.

6. Wash in 3 changes of distilled water.

7. Place in the following solution until the vessels are a golden brown.

Hydroquinone .....	5 gm.
Sodium sulphite .....	10 gm.
Distilled water .....	100 cc.

8. Wash in 4 changes of distilled water.

9. Place in a 1 per cent solution of yellow gold chloride containing 3 drops of glacial acetic acid per 20 cc. until all color disappears from the vessels.

10. Wash in 2 changes of distilled water.

11. Place in a 2 per cent solution of oxalic acid in 1 per cent formalin until the specimens turn a light blue color.

12. Wash in 2 changes of distilled water.

13. Fix in 5 per cent sodium thiosulphate ("hypo") for 5 minutes and then wash in distilled water.

14. Treat with 95 per cent and absolute alcohol and clear in oil of Bergamot. The vessels are then flattened out under the dissecting microscope and mounted in balsam.

#### *B. Fresh Tissue from Animals:*

The brain of the animal should be washed through at first with physiological saline immediately after death until the fluid that comes from the jugular vein is perfectly clear. Then inject 500 cc. of the fixative given in Step 3. After approximately ½ hour the brain should be removed and placed in a fresh solution of the same fixative where it should be left for 24 hours. The vessels are then dissected free from the cortical substance, washed in 3 changes of distilled water, and impregnated as detailed from Step 5.

By the use of this modification it has been possible to impregnate consistently the perivascular nerve supply of intracerebral blood vessels in approximately 100 per cent of specimens examined, as reported in an article<sup>3</sup> to be published shortly. The richness of the vascular nerve supply has been shown to be much greater than before suspected. The fibers are shown uniformly as moniliform structures surrounded by a sheath containing the cells of Schwann.

By variations in the length of time the vessels are left in the protargol solution, and in the time allowed for development in the hydroquinone solution, it is possible to show clearly the smooth muscle cells in the media of the vessels. The minute structure of the muscle cell nuclei showing the depression in the nuclear membrane, termed the diplosome by Maximow,<sup>4</sup> and the condensation of nuclear chromatin material, as described by Boeke,<sup>5</sup> are clearly demonstrated.

### SUMMARY

A method whereby it is possible to demonstrate consistently the perivascular nerve supply of intracerebral blood vessels and the character of the blood vessel wall is presented. The illustrations show the clear picture given by use of this method.

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## DESCRIPTION OF PLATE

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### PLATE 29

FIG. 1. Section showing a perivascular nerve fiber in an intracerebral blood vessel. The moniliform fiber and the sheath of Schwann with nuclei are clearly defined.

FIG. 2. Smooth muscle cell nuclei in the media of intracerebral blood vessels are clearly defined. Diplosomes and nucleoli are well differentiated.



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## ANGIORETICULOENDOTHELIOMA (KAPOSI'S DISEASE) OF THE HEART \*

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"There develop on the skin, without known cause either general or local, brown-red to blue-red nodules of the size of a grain of wheat, a pea or a hazel nut. Their surface is smooth, their consistence densely elastic. Often they are swollen like a sponge filled with blood." So in 1872 wrote Kaposi<sup>81</sup> describing the lesions seen in a disease which has since borne his name. Subsequent study of some 25 cases of the disease enabled him to deduce certain generalizations regarding the entity. The lesions are first observed on the extremities, subsequently they appear on the face and trunk. Spontaneous regression of some of the nodules is a characteristic feature, but no type of treatment ever affords more than temporary relief. Two to 5 years after onset visceral metastases begin and the disease terminates fatally in 3 to 8 years.

Following Kaposi's observations dermatologists throughout the world have studied and recorded cases in which a great variety of manifestations have been observed. On the basis of experience the disease is no longer regarded as invariably fatal and visceral metastases are anticipated in only about 10 per cent of cases. Microscopic examination of the lesions has led to fairly general agreement as to the histological features necessary for establishment of the diagnosis. Nevertheless, there is still but little agreement regarding the etiology and pathogenesis of the condition.

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TABLE I

*Digest of Cases Reported in Literature Since 1932*

Author	Number of cases	Sex	Age	Race or nationality	Occupation	Duration of lesion	Location of lesion	Microscopic diagnosis	Special features	Animal inoculation	Group	Theory of nature of disease
Andrews <sup>1</sup>	1	M	yrs. 19	Mulatto	Truck driver	10 wks.	Skin	(1) Fibrosarcoma (2) Kaposi Kaposi Kaposi				Not stated
Arias <sup>2</sup>	1	M	49	Spanish		6 mos.	Skin	Kaposi			I	Not stated
Barringer and Dean <sup>4</sup>	2	M	45			— mos.	Skin and penis	Kaposi				"This case supports neuro-origin"
Bilancioni <sup>5</sup>	1	M	56 62	Italian Italian	Merchant	2 mos. 12 yrs.	Skin and penis Skin and larynx	Kaposi Kaposi			I	Systemic vascular disease with neoformation of caps.
Caripino and Secchi <sup>7</sup>	1	M	58	Italian	Sponge fisher	8 mos.	Skin	Kaposi	Antecedent fibrosarcoma. died		I	"More neoplastic than a granuloma"
Casabianca <i>et al.</i> <sup>8</sup>	1	M	52	Corsican	Sailor	6 mos.	Skin	Kaposi			4	Reticuloendothelial hyperplasia
Van Cleve and Hellwig <sup>10</sup>	1	M	58	American	Painter	2 yrs.	Skin, liver, kidneys, stomach, intestine, pericardium	Kaposi	1st lesion nodule in groin, died		3	Granuloma becomes neoplastic
Denzer and Leopold <sup>12</sup>	1	M	4	Italian		1 yr.	Skin, widespread, internal	Kaposi	Died			Not stated
Dupont <sup>14</sup>	12		Case histories not given					Kaposi	20 biopsies		4	Angioreticulosis <i>cf.</i> von Recklinghausen's neurofibromatosis
Eljasz <sup>15</sup>	1	M	76	Polish Jew		17 yrs.	Skin and bladder	Kaposi	Cornu cutaneum superimposed			Not stated
Ellis <sup>16</sup>	1	M	40	American negro		2 yrs.	Skin	Kaposi	In blood smear 46% lymphocytes			Not stated
Frölich <sup>18</sup>	1	M	44	Polish Jew	Ditch digger	1½ yrs.	Skin	Kaposi			4	Reticuloendothelial disease

Goldschlag <sup>19</sup>	1	M	65	Russian Jew	Merchant	3½ yrs.	Skin and viscera (?)	Kaposi	Antecedent lymphogranuloma	4	Reticuloendothelial disease
Gonzales and Vidaurreta <sup>20</sup>	1	M	55	Italian		1½ yrs.	Skin	Kaposi	Died	1	Angiosarcoma
Gougerot <i>et al.</i> <sup>21</sup>	1	F	57	Armenian		9 yrs.	Skin	Kaposi			Not stated
Gougerot <i>et al.</i> <sup>22</sup>	1	M	39		Wine merchant	2 mos.	Skin and mouth	Kaposi			Not stated
Greppi and Bettoni <sup>23</sup>	1	M	40			1 yr.	Penis, lymph node, psoas, lung	Kaposi	Hemolytic splenomegaly, died	4	Chronic reticuloendotheliosis
Grzybowski <sup>23</sup>	3	M	54	Jew		12 yrs.	Skin		Died	1	Neoformation of blood vessels with endothelial hyperplasia
Guccione <sup>24</sup>	2	F M M	46 63 56	Jew Jew		16-20 yrs. 6 mos.	Skin Skin and tongue Skin	Kaposi Kaposi	Died	4	Angioreticulosis with possible endocrine basis
Homma <sup>25</sup>	1	M	63			1 yr.	Skin	Kaposi			
Jessup <sup>26</sup>	1	M	70	Italian	Laborer	10 yrs.	Skin	Kaposi			Specific infection
Kusnezow <sup>26</sup>	1	M	65	Caucasian		11 mos.	Skin	Kaposi		2	Not stated
	1	M	32			2 yrs.	Skin, tongue, trachea, intestine	Kaposi	In blood smear 55% lymphocytes, died		Visceral lesions not metastases
Lane and Greenwood <sup>23</sup>	1	M	42	Italian	Laborer	3 yrs.	Skin	Kaposi	Also lymphatic leukemia and mycosis fungoides, died		Possible common etiological factor for all

TABLE I (Continued)

Author	Number of cases	Sex	Age yrs.	Race or nationality	Occupation	Duration of lesion	Location of lesion	Microscopic diagnosis	Special features	Animal inoculation	Group	Theory of nature of disease
Lang and Haslhofer <sup>28</sup>	2	F	58		No further data			Kaposi			I	Generalized hemangiomas with anomaly in arrangement of vascular system
Leigh <sup>27</sup>	1	M	51	Italian	No further data	1 mo.	Skin	Kaposi	Cardiovascular renal disease		Miscellaneous	"Affection of vascular system"
Mierzecki <sup>29</sup>	7	M	26	Jew		2 yrs.	Skin, lymph node, lungs, kidney				I	Starts as neoplasm, ends as simple granulation tissue
		M	62	Jew		Many yrs.	Skin					
		M	29	Jew		6 mos.	Skin	Kaposi				
		M	65	Greek catholic	Carpenter	2 yrs.	Skin					
		M	74	Greek catholic	Watchman	3 yrs.	Skin					
		M	62	Jew	Woodman	2 yrs.	Skin					
		M	46	Roman catholic	Bricklayer	24 yrs.	Skin and bones	Kaposi				
Pack <sup>32</sup>	1	M	72	Russian Jew		3 yrs.	Skin	Kaposi		Reinoculation negative	I	Glomus type of tumor
Pardo Castello <sup>31</sup>	1	M		Negro	(Quoted by Ellis)					No further data		Not quoted
Pearce and Walker <sup>30</sup>	1	M	50			6 mos.	Skin, gum, genitalia	Kaposi	Died		I	Sarcoma
Pitotti <sup>37</sup>	1	M	17	Italian		2 yrs.	Skin	Kaposi				Not stated

Roger and Vigne <sup>48</sup>	1	M	50	Corsican	Navigator	9 yrs.	Skin, lips, genitalia	Kaposi	Von Recklinghausen's disease in bones, died, no autopsy	Anterior chamber eye of guinea pig negative	Miscellaneous	Not stated
Santori <sup>49</sup>	4	M M	50 59	Italian Italian		1 yr. 3 yrs.	Skin Skin	Kaposi Kaposi				"Systemic vascular disease"
Schirmunkaja and Tschotschia <sup>50</sup>	1	M	70	Italian		25 yrs.	Skin	Kaposi	Died, no autopsy			Benign tumor
Szary and Bardin <sup>51</sup>	1	M	22 80	Russian	Clerk	5 yrs. 4 yrs.	Skin, pharynx Skin	Kaposi Kaposi	Original diagnosis von Recklinghausen's disease		1	Not stated
Vigne and Pédat <sup>52</sup> Webster <sup>53</sup>	1 1	M M	63 77	Corsican Polish Jew	Docker	2½ yrs. 6 yrs.	Skin Skin	Kaposi Kaposi	Died of pneumonia, no autopsy			Not stated Not stated
Cholisser and Ramsey	2	M M	26 30	American American	Clerk Policeman	2 mos. 1 mo.	Heart, lungs, liver, pericardium Heart	Kaposi Kaposi	Died	Negative	4	Angioreticuloendothelioma Angioreticuloendothelioma

For this reason it is important that each new case encountered be regarded not only on its merits but also in the light of other cases reported in the literature. Two cases which we have recently been privileged to study clinically and at postmortem present certain features not previously described and stress the fact that Kaposi's disease is one of general medical importance as well as of dermatological significance.

### REVIEW OF THE LITERATURE

Two recent monographs have dealt exhaustively with the older literature on the subject and for our present purposes it is sufficient to adopt the statistics compiled by their authors rather than to refer directly to original reports, each dealing with but a few isolated cases. Dörffel<sup>13</sup> examined the literature up to 1932 in connection with a report of 16 cases which had come under his own observation. Kren<sup>33</sup> wrote the chapter on Kaposi's disease in Jadassohn's *Handbuch der Haut- und Geschlechtskrankheiten* in the following year. This very detailed treatise provides a complete bibliography and thorough analysis of all cases reported and all theories pertaining to etiology, as well as discussions of the pertinent clinical and therapeutic problems.

The literature subsequent to 1932 has been reviewed by the present writers and the figures gleaned from it are added to those of Dörffel and Kren to form the basis of Tables I, II and III, and Chart 1, which carry the statistics down to the end of the year 1937. Data from the 2 cases herewith reported are also included in the statistical summaries.

*Age Incidence:* Dörffel gathered 356 cases from the literature and added 16 of his own. Of this total of 372 cases the age was stated in 234. Our review of subsequently published cases has added 60 to the total, in 47 of which the age is known. The age in the 2 cases herewith reported is also known. Chart 1 shows the age distribution, by decades, of the total 283 cases. The peak of incidence in the 5th, 6th and 7th decades has been recognized as a characteristic finding throughout the history of the disease.

*Sex:* Dörffel counted 21 females among the 356 cases in the literature, and 2 of his own cases occurred in women. Kren considers this figure too low as his count of "nearly 500 cases" included "about 40 females." Among the cases recorded since 1932

we found 3 women. Those herewith reported occurred in men. In our series from the literature, as in Dörffel's, the sex of the patients is not always ascertainable. Combining the known cases from both series, the ratio of male to female is found to be 385:26.

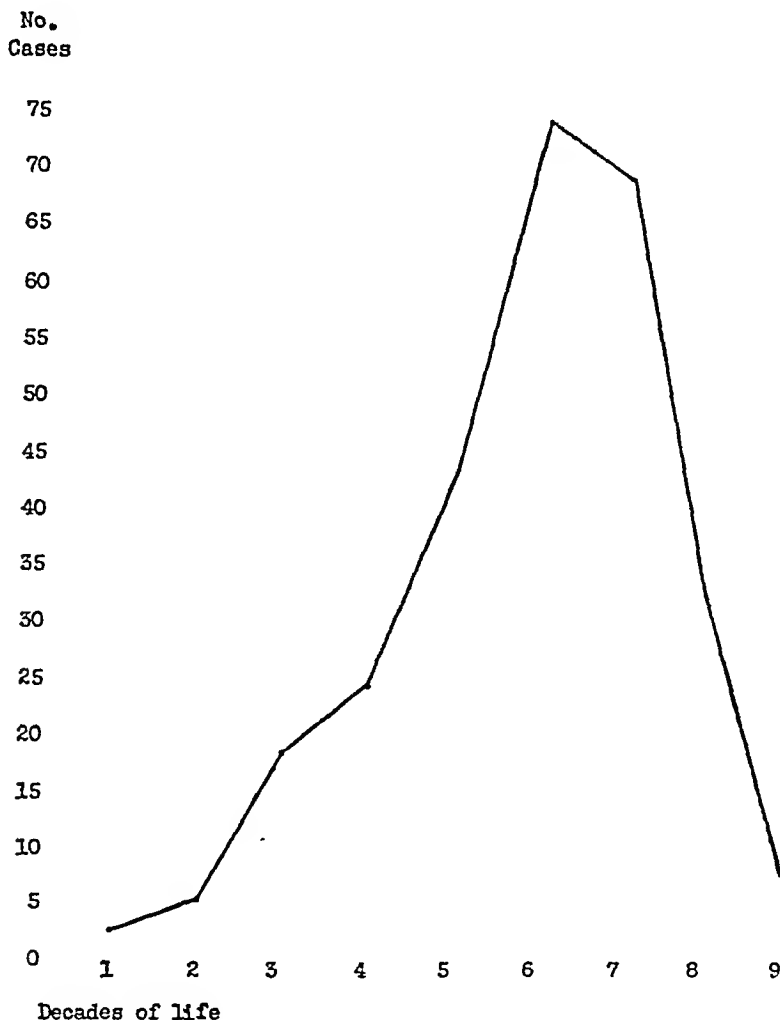


Chart I

Age Incidence of Kaposi's Disease  
Total 434 Cases

Thus, women represent 5.99 per cent of the (uncorrected) total of 434.

*Race:* The racial stock or nationality of the patient is given in 40 of the 62 recent cases (including our own 2). The distribution is as follows: 12 Italians, 12 Jews (2 Russian, 3 Polish, 7 unspecified), 3 Corsicans, 3 Americans, 2 negroes (1 Cuban, 1 American), 1 "North Caucasian," 1 mulatto, 1 Spanish, 1 Armenian,

1 Russian, 2 "Greek catholics," 1 "Roman catholic." This is in line with Dörrfel's opinion that the disease is more closely linked with geographic than with racial origin, Eastern Europe and Northern Italy being the areas where most of the cases originate, though no part of the world lacks representation, with the possible exception of China and Japan (Mainta, quoted by Kren).

*Occupation:* The patient's occupation is recorded only 17 times in recent reports. Outdoor labor is noted 13 times (truck driver, painter, laborer, sailor, fisherman, carpenter), indoor work 4 times (clerk, merchant, wine merchant). In addition, of our 2 patients 1 was a policeman and the other a mailing clerk. This agrees with

TABLE II  
*Location of Visceral Lesions as Recorded in Literature Prior to 1932*

Most common (in order)	Frequent	Uncommon	Rare	Never recorded
Gastro-intestinal tract, all portions	Faucial tonsil	Spleen	Heart	Brain
Liver	Bones	Pancreas	Pericardium	Thyroid
Lungs	Larynx	Kidneys	Central nervous system	Ovary
Lymph nodes	Conjunctiva	Adrenals	Peripheral nerves	Uterus
retroperitoneal		Peritoneum		
mesenteric		Testes	Tongue	
		Epididymis	Bladder	
		Trachea	Muscles	
		Bronchi	Pituitary	
		Pleura		

Dörrfel's and Kren's finding that office workers and professional men are less frequently affected than laborers and out-of-door workers. The discrepancy is more pronounced in their larger series.

*Duration of Disease:* The cases in the recent literature all fall within the general limits noted by Dörrfel, namely, 8 months to 25 years where the disease was fatal. Six months to 2 years is the spread in the 13 fatal cases recorded in the literature since 1932. Our own 2 patients form an exception. One was ill for only 1 month and the other for 2 months.

*Distribution of Visceral Lesions:* Most authors take particular care to avoid the use of the term "metastasis" in this connection because of its specific connotation. Since there is no general agreement that the disease is truly a malignant neoplasm, it would be undesirable to designate the visceral lesions as metastatic. The accompanying table (Table II), an adaptation of summaries pre-

sented by Dörffel and Kren, shows the relative frequency of involvement of various viscera as reported in the earlier literature. For the most part the statistics are based on positive autopsy findings, although hemorrhages from the orifices in patients with cutaneous lesions were sometimes accepted as proof of visceral involvement. Table III shows the distribution of visceral lesions reported since 1932, including the cases herewith described. In this series, autopsy or biopsy findings confirmed the clinical diagnosis in every case except the one of urinary bladder involvement in which the diagnosis rested on cystoscopic examination of a patient whose skin lesion was examined microscopically.

TABLE III

*Location of Visceral Lesions as Recorded in Literature Since 1932  
(Authors' own cases included)*

Location	Number of times	Location	Number of times
Penis .....	3	"Widespread" (not specified) ..	2
Oral cavity .....	3	Heart .....	2
Upper respiratory tract .....	3	Tongue .....	2
Lymph nodes .....	3	Pericardium .....	2
Lung .....	3	Tonsil .....	1
Stomach .....	2	Bladder .....	1
Intestines .....	2	Diaphragm .....	1
External genitalia (male) .....	2	Psoas muscle .....	1
Liver .....	2	Bones .....	1
Kidney .....	2		

Up to the time of Dörffel's and Kren's articles no case had been reported in which skin lesions were lacking, and only in Paolini's case <sup>43</sup> did a visceral lesion antedate the cutaneous manifestations. The literature since 1932 includes the report of 1 patient (that of Greppi and Bettoni <sup>23</sup>) in whom no skin lesion ever appeared during the year of illness prior to death. The primary lesion occurred upon the glans penis, the inguinal lymph nodes became involved, and at autopsy there was extension into the psoas muscle and nodules were found in the lung. Four reports in recent years have dealt with cases in which non-cutaneous manifestations preceded skin lesions. Pearce and Valker's <sup>46</sup> patient first noted a nodule on his gum several months before his skin became involved. Barringer and Dean <sup>4</sup> twice noted primary lesions on the glans penis. Van Cleve and Hellwig, <sup>10</sup> and Goldschlag <sup>19</sup> reported cases



in which the inguinal lymph nodes were the primary seat of the disease. In Goldschlag's case the original lymph node involvement was diagnosed lymphogranuloma inguinale, but he looked on this as an "ungewöhnlichem Vorstadium" of Kaposi's disease. The cases herewith reported lacked skin lesions and were primary in the right auricle of the heart.

*Theories of Origin:* There are nearly as many theories regarding the origin of this disease as there are investigators in the field. Briefly, the opinions may be classified in four major groups: (1) neoplasm; (2) infectious granuloma; (3) infectious granuloma with neoplastic potentialities; and (4) reticuloendothelial hyperplasia. For an exhaustive treatment of this aspect of the study of the disease the reader is referred to Kren's article. In the present connection it is desirable to mention only the most important subheadings in these four main groups to supply the necessary background for evaluation of recent opinions.

(1) Adherents of the theory of neoplasia are divided as to whether the tumor is benign or malignant and as to the origin and nature of the tumor cells. The nomenclature proposed indicates some of the opinions current on these points (Table IV).

(2) Proponents of the infectious granuloma theory have in their favor the natural history of the disease which so frequently includes spontaneous regressions which would be hard to reconcile with a malignant neoplasm. To their disadvantage stands the fact that no etiological agent has ever been demonstrated despite extensive bacteriological work, and that transfer of the disease to animals has never been definitely established, although attempts have been made using every common laboratory animal and bird. Reinoculation of patients with their own tumor tissue has also failed to give positive results (Pack <sup>42</sup>).

(3) The existence of certain "predisposing causes" and "exciting factors" is postulated by various authors to explain a malignant change in a previously benign growth, a neoplastic transformation on the part of an infectious granuloma or the development of reticuloendothelial hyperplasia. These include trauma, chilling or freezing of affected parts, alcoholism, drug sensitivity, erysipelas, cellulitis, arteriosclerosis, syphilis and acute systemic diseases, such as cholera, malaria, grippe and pneumonia.

(4) Those who consider the condition merely a reticuloendo-

thelial hyperplasia note not only the suggestive histological picture of the disease and the simultaneous occurrence of widely scattered lesions, but also the not uncommon occurrence of other stigmata of disease of the reticuloendothelial system in patients suffering from Kaposi's disease. Cases have been recorded in which the patient showed variations from mild lymphocytosis to

TABLE IV

*Sarcoma Idiopathicum Multiplex Haemorrhagicum (Kaposi, 1872)*

*Synonyms*

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1.	Angiosarcoma peritheliale fusocellulare
2.	Angiosarcoma pigmentosum
3.	Primitives haemorrhagisches acrosarcoid
4.	Acrosarcoma multiplex cutaneum telangiectoides
5.	Acroangioma hemorrhagicum
6.	Sarcomatosis cutanea idiopathica
7.	Sarcomatosis primitiva telangiectosica
8.	Granuloma multiplex hemorrhagicum
9.	Sarcoma cutaneum teleangiectaticum multiplex
10.	Angioendothelioma cutaneum
11.	Sarcoma haemangioendotheliale intravasculare
12.	Haemangioendothelioma cutaneum
13.	Sarcomatosis teleangiectaticum cutanea idiopathica generalisata
14.	Perithelioma multiplex nodulosum cavernosum lymphangiectoides cutaneum
15.	Acroperithelioma idiopathicum multiplex cavernosum lymphangiectoides cutaneum
16.	Pseudosarcomatosis teleangiectaticum Kaposi
17.	Sarcomatosis multiple hemorrhagica y pigmentaris tipo Kaposi
18.	Sarcomatosi cutanea multipla emorragica di Kaposi
19.	Multiple idiopathic hemorrhagic sarcoma of Kaposi
20.	Angiosarcoma multiplex
21.	Angiosarcoma endotheliale
22.	Granuloma angiomatoides
23.	Sarcoid Kaposi
24.	Acrosarcoma Kaposi
25.	Systematisierte angiomatosis
26.	Kaposi's sarcoma
27.	Kaposi's disease
28.	Angioreticuloendothelioma

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frank lymphatic leukemia, mycosis fungoides and lymphogranuloma inguinale.<sup>11, 16, 19, 23, 34, 35, 55</sup> The sites in which lesions of Kaposi's disease are found further support the hypothesis that it is of reticuloendothelial origin, since the regions most richly supplied with such tissue are most commonly affected.

The opinions of recent investigators on the disputed question of the origin and nature of Kaposi's disease are recorded in the last column of Table I. The numerical notations in the column headed

"Group" in that table refer to the four general groups noted above.

### CASE HISTORIES

CASE 1. A white male, 26 years of age, a mailing clerk, entered the hospital Nov. 8, 1937, complaining of generalized weakness, shortness of breath, and pain in the left arm. He had lived in the city of Washington, D. C. all his life. The family history and previous history were non-contributory. Two months before admission he had had a week's illness which was diagnosed acute influenza. Upon recovery he went back to work for 3 days but was forced to return to bed because of weakness and dyspnea. These symptoms became progressively more severe. Night sweats occurred and he developed a persistent pain in the left arm which radiated from the axilla to the finger tips.

*Physical Examination:* On admission the physical examination revealed a well developed man, acutely ill, showing dyspnea and moderate cyanosis. The temperature was 99° F., the pulse 120, and the respiratory rate 20. The blood pressure was 115/90 mm. Hg. The neck was short and thick and the great vessels were distended. The thorax was symmetrical, with equal expansion on both sides and no bulging of the intercostal spaces. The lung resonance was normal bilaterally. The heart was markedly enlarged but there was no evident pulsation in the precordial or great vessel areas. Sounds were distant and without murmurs. The liver extended three fingers' breadths below the costal margin and was smooth and tender. The spleen and superficial lymph nodes were not palpable. The skin over the entire body was moist and dusky, without eruptions or excoriations.

*Laboratory Examinations:* The urinalysis was essentially normal. The blood count showed: white blood cells 5150, 51 per cent segmented forms, 18 per cent band forms, 31 per cent lymphocytes; red blood corpuscles 3,900,000; and hemoglobin 70 per cent (Dare). A non-protein nitrogen determination was 46.5 mg. per cent. Roentgenological examination of the chest 3 days after admission showed some suggestion of fluid in the right pleural cavity with the heart enlarged to the left.

The patient's temperature ranged between 99° and 101° F. during his hospital course. His condition became progressively worse, with increasing dyspnea and cyanosis. His blood pressure was recorded as 120/0 mm. Hg. shortly before death on the 6th hospital day.

### *Postmortem Examination*

The body was well nourished. There was cyanosis of the head and neck and slight generalized icterus. The skin was moist and showed no eruptions or gross lesions.

The pathological changes were confined to the heart and pericardium, pleura, diaphragm and liver. The general relationships of the thoracic viscera were distorted by the enormous enlargement of the heart and the presence of 2000 cc. of bloody fluid in the right pleural cavity. The right lung was partially collapsed.

The visceral and parietal pleurae showed numerous, densely adherent, soft hemorrhagic nodules which varied from 0.5 to 2 cm. in diameter. Similar nodules were present on the superior surface of the diaphragm. The left lung and pleural cavity showed nothing unusual. The mediastinal lymph nodes were markedly enlarged and hemorrhagic, and were incarcerated in a dense neoplastic mass which surrounded all the great vessels. The pericardium was thickened and the pericardial cavity obliterated by a similar neoplastic growth which varied from 2 to 3 cm. in thickness. The right auricle was markedly distended. Section revealed a tumor nodule originating in the wall and projecting into the auricular cavity. The endothelium was unbroken. The upper portion of the mass was continuous with the mediastinal lymph nodes. The nodule within the auricular cavity measured 6 cm. in diameter and practically occluded the tricuspid orifice. The valve leaflets were not involved. The right ventricle was collapsed; its wall was invaded to a moderate extent by the neoplasm from the pericardial cavity. The left auricle and ventricle both showed a similar neoplastic involvement. The pulmonary, mitral and aortic valves were unchanged though the aortic ring was definitely compressed by the surrounding tumor. The appearance of the neoplasm varied markedly in different portions. White mucoid areas alternated with large irregular blood sinuses, some of which were filled with bright red fluid blood while others showed areas of degeneration and were filled with a soft chocolate brown substance (Fig. 1).

The liver weighed 2070 gm. and showed advanced chronic passive congestion. On the superficial surface of the right lobe was a hemorrhagic nodule, 3 cm. in diameter, which somewhat resembled an area of infarction (Fig. 2). A similar nodule was noted in the quadrate lobe and two smaller ones in the central portion of the left lobe. In the cortex of each kidney were several, small, irregular hemorrhagic areas which also looked not unlike recent infarcts. The omentum was studded with small hemorrhagic nodules 2-3 mm. in diameter. Some of these were soft in consistence, others granular. Several similar nodules were present in the tissues surrounding the duodenum and jejunum. These did not communicate with the wall of the intestine. Examination of the entire gastro-intestinal tract revealed moderate congestion but no evidence of ulceration, neoplasia or obstruction.

*Anatomical Diagnoses:* Hemorrhagic neoplasm of the heart, with secondary nodules in the pericardium, mediastinum, pleura and liver; right hemothorax; hemorrhagic infarcts of the kidneys; and advanced passive congestion of the liver.

CASE 2. A white male, 30 years of age, a policeman, entered the hospital Dec. 26, 1937 complaining of weakness, shortness of breath, and tenderness in the upper right quadrant. He was born in the state of Oklahoma, but had resided in the city of Washington, D. C. for several years. The family history and previous history were non-contributory. In the early part of November he was in bed for a few days with a severe cold and bronchitis. On recovery he went back to work although he complained of being quite weak. On December 14th he returned to bed because of nausea and vomiting and tenderness in the upper right quadrant. His attending physician noted also a painless swelling of the patient's neck. The above symptoms subsided somewhat under bed rest but progressive dyspnea developed.

*Physical Examination:* On admission the physical examination revealed a well nourished man, acutely ill, with evident orthopnea and moderate cyanosis. The temperature was 99° F., the pulse 85, and the respiratory rate 20. The blood pressure was 110/0 in the right arm, 120/0 in the left arm. The neck was short, thick, and markedly congested. The thorax was of the hypersthenic type, symmetrical and with equal expansion on both sides. Lung resonance was good bilaterally. The heart was markedly enlarged both to the right and to the left. The point of maximum impulse was not palpable. The sounds were distant, weak and without murmurs. The abdomen was tender in the upper right quadrant and the liver palpable three fingers' breadths below the costal margin. The spleen and superficial lymph nodes were not palpable. The skin was moist and cyanotic. There were no lesions present.

*Laboratory Examinations:* Urinalysis was essentially normal. The blood count showed: white blood cells 13,300, 58 per cent segmented forms, 4 per cent band forms, 38 per cent lymphocytes; red blood corpuscles 4,300,000; hemoglobin 84 per cent (Dare). Roentgenological examination of the chest on December 31st revealed massive pericardial effusion.

Paracentesis of the pericardium was performed on December 31st, 720 cc. of bloody fluid being withdrawn. The fluid showed no growth on culture after 72 hours of incubation. The patient's dyspnea and cyanosis became more pronounced after the paracentesis and death occurred 2 hours later.

### *Postmortem Examination*

The body was well nourished. The skin was cyanotic, especially in the region of the head and neck. The latter was short, stout, and appeared distended but not edematous. The superficial vessels of this region were markedly dilated and engorged. No cutaneous lesions of any sort were noted.

The essential pathological changes were confined to the heart, pericardial cavity and liver. The general relationships of the thoracic viscera were somewhat distorted. The pericardial sac

was distended by 1000 cc. of bloody fluid and the heart was displaced to the left. The right pleural cavity contained 1200 cc. of bloody fluid; the left 800 cc. Both lungs were partially collapsed and showed areas of atelectasis and emphysema, but no pneumonic, tuberculous or neoplastic involvement. The mediastinal lymph nodes were moderately enlarged but on section showed only anthracosis and congestion. The heart weighed 750 gm. The anterior surface consisted almost entirely of the right auricle, which measured 10 by 12 cm. in its oblique diameters. On opening the auricle a firm, compact tumor mass was found growing from the anterior wall into the auricular cavity which it practically obliterated. The tumor almost completely blocked the junction of the superior and inferior venae cavae and caused a ball valve type of obstruction at the tricuspid orifice. The endothelial lining of the auricle was smooth and unbroken (Fig. 3). On section through the tumor it was found to consist of a semisolid fleshy substance which varied in color and consistence in different portions. Centrally the tumor was soft, white and mucoid; at the periphery it was dense and reddish brown in color. A few hemorrhagic extravasations were noted in the upper portion, while on the auricular surface were present large blood sinuses, some containing bright red fluid blood and others a chocolate brown substance. There was no extension of the tumor into the left auricle or below the auriculoventricular junction. The right ventricle was partially collapsed, its wall moderately hypertrophied and toxic. The tricuspid and pulmonary valves showed nothing unusual. The left auricle and ventricle contained a small amount of clotted blood. Their walls were of normal thickness, color and consistence. The mitral valve was smooth and glistening. The aortic valve, while moderately compressed, was otherwise unchanged.

The walls of the various portions of the gastro-intestinal tract were thin. There was evidence of moderate passive congestion but no lymphoid hyperplasia or neoplastic involvement. The liver weighed 1850 gm. Externally and on cut section it showed marked chronic passive congestion with no gross or microscopic evidence of neoplasia.

*Anatomical Diagnoses:* Angioreticuloendothelioma (Kaposi's disease) of the heart; hemopericardium; bilateral hemothorax; and advanced passive congestion of the liver.

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*Anatomical Diagnoses:* Angioreticuloendothelioma (Kaposi's disease) of the heart; hemopericardium; bilateral hemothorax; and advanced passive congestion of the liver.



### *Microscopic Examination*

Microscopic examination of hematoxylin-eosin stained sections from the primary tumors of the auricles in both cases and of the secondary nodules in the 1st case shows the neoplasms to be cytologically identical (Figs. 4 and 5). The disordered architectural arrangement and the multiplicity of cellular infiltrations and proliferations at once attract attention (Fig. 6). The structure in different portions is as variable as is the pleomorphism in individual regions. One obvious feature common to all sections is vascularity. In some areas the tumor is made up almost entirely of small, newly formed blood vessels with thin walls consisting only of endothelium resting on a reticular base. In other places large blood sinuses are present. These likewise possess thin walls which are frequently broken, permitting hemorrhagic extravasation. Some of the vessels are engorged with fresh blood while others are filled with degenerated debris and surrounded by massive irregular areas of liquefactive necrosis. Small areas of polymorphonuclear leukocytic and dense lymphocytic infiltration are dispersed throughout. The more solid portions of the tumor consist of proliferated embryonic spindle cells which vary in size, shape and arrangement. Some are small and closely packed together; others are large, almost oval, and possess hyperchromatic nuclei with an occasional mitotic figure (Fig. 7). Broad sheets of spindle cells invade the myocardium, resembling a rapidly growing fibrosarcoma (Fig. 8). In other places the cells appear more differentiated and assume a somewhat "windblown" appearance, or abruptly form irregular whorls suggestive of neurogenic origin (Fig. 9). Small areas of mucoid degeneration are found in the deeper portions of the tumor nodules.

The appearance of the predominating spindle cell suggests that it might have a fibroblastic origin, but sections stained by van Gieson's and Mallory's connective tissue stains show only the faintest trace of true connective tissue. Bielschowsky reticulum stains, however, show diffuse, massive reticular proliferation. In some areas the reticulum is fine, lace-like and compact (Fig. 10). In others, long thick processes are found which present a "broken twig" appearance similar to that seen in so-called Hodgkin's sarcoma (Fig. 11). The reticular arrangement around the blood

vessels is of interest. Each vascular space, whether of the capillary or the sinus type, is completely enveloped by reticulum which clearly outlines its wall (Fig. 12). In the areas of hemorrhagic extravasation a mosaic of fine reticular elements is attempting enclosure of the blood. In the myocardium and the liver, reticular proliferation precedes the infiltration of embryonic spindle cells. Sections stained by the Giemsa, the Gram-Weigert and the Ziehl-Neelsen methods reveal no bacteria, and Levaditi stained sections are negative for treponemas.

The tumors from the 2 cases are histologically identical with respect to both architectural arrangement and cytology and correspond in every way to the hemorrhagic sarcoma of Kaposi.

Morcellated portions of fresh tumor tissue from both cases and Berkefeld filtrates of such preparations were injected by various routes — subcutaneously, intraperitoneally, intravenously and intracerebrally — into a number of experimental animals including rabbits, mice, guinea pigs and canary birds. A localized pyogenic abscess developed in 1 rabbit at the site of the subcutaneous inoculation. With this exception no local or systemic reaction has been seen in any case up to the present time (151 and 178 days respectively since inoculation).

### DISCUSSION

The unusual features presented by the 2 cases herewith reported may be summarized as follows: (1) the comparative youth of the patients; (2) the fact that both of them were native-born Americans and one of them was an indoor worker; (3) the rapidly fatal course of the disease in both instances, which obviously was the result of primary cardiac involvement; (4) the unusual site of the primary lesion in the right auricle in both patients; and (5) the absence of skin lesions in both cases.

In our review of the literature of Kaposi's disease we were unable to find a single instance in which the condition was primary in the heart. Reviewing the literature on primary cardiac neoplasms, however, we did find 8 instances,<sup>3, 6, 24, 27, 41, 52, 53, 57</sup> in which the descriptions of the gross and microscopic appearance of the tumor strongly suggested that the conditions were similar to those in the cases herewith reported. Through the courtesy of Dr. T. B. Mallory we were able to examine the microscopic prepa-

ration from Case No. 22491,<sup>6</sup> which proved to be identical with our own cases. Thus it is evident that primary localization of Kaposi's disease in the heart is not as rare as would at first appear, the difficulty heretofore having been in its identification.

Of particular interest in the cases reported here is the fact that we were unable to study the cytology of the lesion with especial thoroughness and to attempt transmission of the tumor to animals of many sorts and by various routes.

The results of these studies have given us a better insight into the nature and origin of these tumors. To the question, "Is Kaposi's disease an infectious granuloma?" it is our opinion that a negative answer should be given. With the single exception of Justus,<sup>39</sup> who in 1909 published a brief undocumented note on his animal experimentation, no student of the disease has been able to date to transmit it to lower animals. Pack<sup>42</sup> even attempted reinoculation of a patient with his own tumor without success. The results of our own particularly intensive attempts at transmission to animals are in line with these previous experiences. In summary, the work on this aspect of the disease may be said to suggest very strongly that the tumor is not a granuloma. Coupling this evidence with the grossly invasive character of the growth and the striking appearance of malignancy exhibited by microscopic preparations of the tumor, we have concluded that the disease is truly neoplastic. The "reticuloendothelial hyperplasia" theory seems as untenable as the "granuloma" theory because of the invasive character of the growth.

With respect to the specific type of neoplasm represented by the Kaposi tumor it becomes necessary to decide what tissue of the body could give rise to the numerous and diverse structures observed — newly formed blood vessels, including endothelium and adventitial connective tissue, embryonic spindle cells, lymphoid elements and well organized reticulum. Only from the reticuloendothelial system could all these types be derived. The tumor should therefore be given the name "reticuloendothelioma" to show this relationship. Since the neoformation of blood vessels is so outstanding a characteristic, it seems desirable to add the prefix "angio" to distinguish this tumor from other reticuloendothelial neoplasms in which other components predominate. As eponyms are at best undesirable makeshifts chiefly employed when

the true nature of a condition is obscure, and inasmuch as the disease Kaposi originally described was characteristically a skin disease, it seems desirable to use the nosological term "angio-reticuloendothelioma" for this tumor at all times, save when the distinctive name "Kaposi's disease" may be applied specifically to the skin manifestations.

### SUMMARY AND CONCLUSIONS

The problem of the origin and nature of the lesions of Kaposi's disease has been reanalyzed on the basis of a review of the literature on the subject and in the light of a thorough study of 2 recent cases in both of which the disease was primary in the right auricle of the heart and in which skin lesions were lacking.

It is concluded that the condition is a true neoplasm derived from the reticuloendothelial system with neoformation of blood vessels a prominent distinguishing characteristic.

Since tumors are properly named with respect to their tissues of origin, it is proposed that in the future the scientific term "angioreticuloendothelioma" be used in preference to the term "Kaposi's disease."

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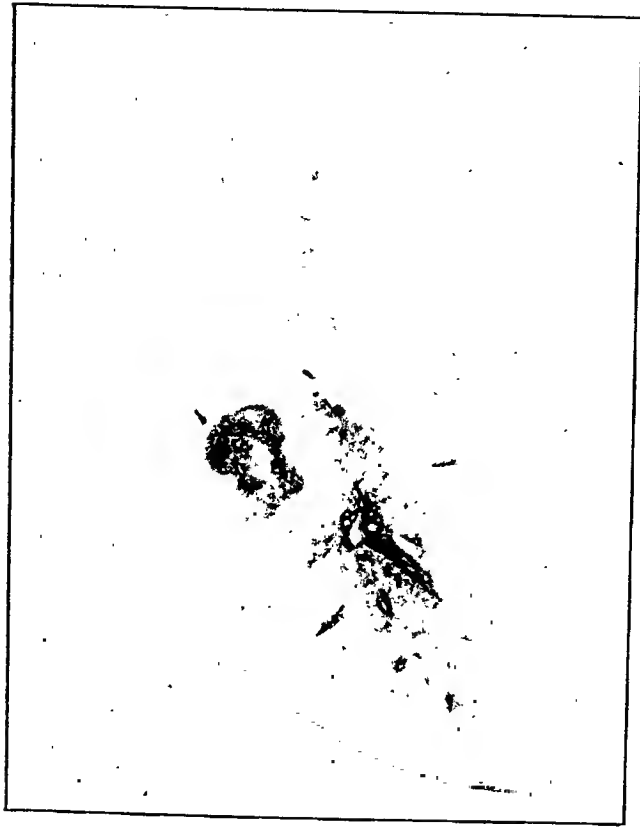


## DESCRIPTION OF PLATES

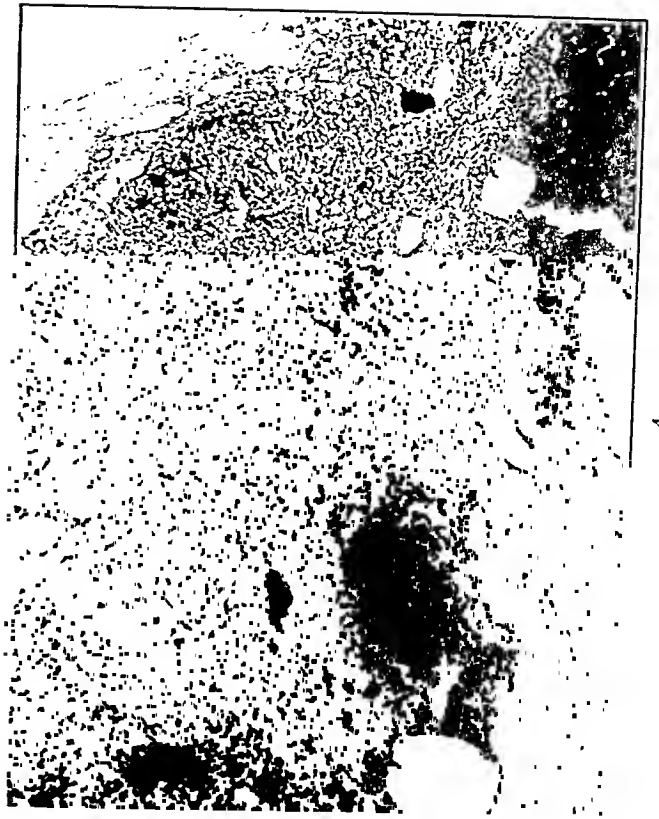
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### PLATE 30

- FIG. 1. Case 1. Sagittal section of heart showing tumor filling right auricle and obliteration of pericardial space by neoplastic tissue.
- FIG. 2. Case 1. Section of liver showing tumor nodule in right lobe.
- FIG. 3. Case 2. Heart opened to show tumor mass filling right auricle. The unbroken endothelium of the auricle covers the nodule.
- FIG. 4. Case 1. Microphotograph showing general character of the neoplasm, including proliferation of embryonic spindle cells and formation of new capillaries and blood sinuses. Hematoxylin-eosin stain.  $\times 45$ .



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Choisser and Ramsey

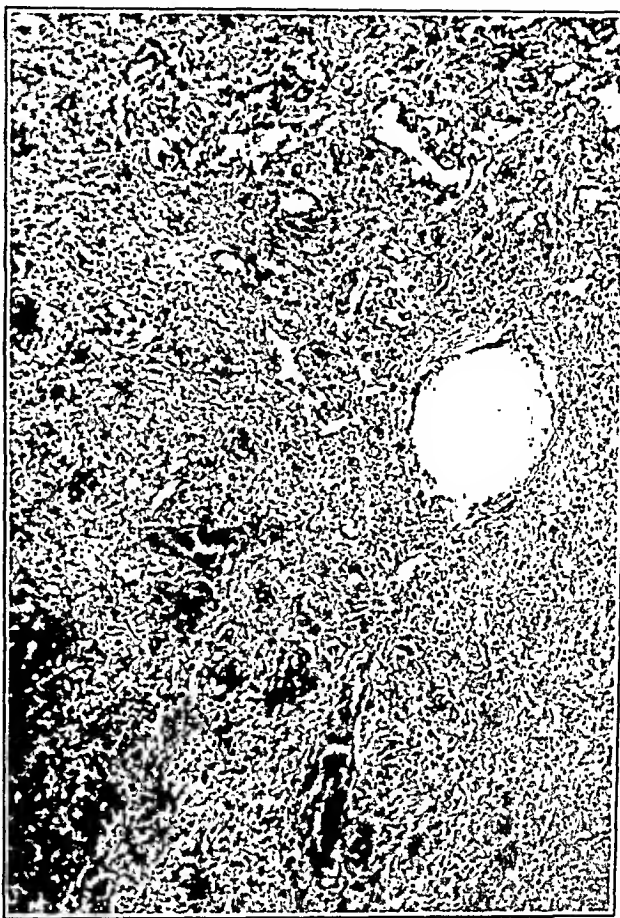
Angioreticuloendothelioma of the Heart

PLATE 31

- FIG. 5. Case 2. Microphotograph showing general character of neoplasm and illustrating particularly the similarity between the microscopic appearances of the two tumors. (See Fig. 4.) Hematoxylin-eosin stain.  $\times 45$ .
- FIG. 6. Case 1. Microphotograph showing multiplicity of cellular types. Small thin-walled, newly formed blood vessels are seen below and a large blood sinus above. Hematoxylin-eosin stain.  $\times 150$ .
- FIG. 7. Case 2. Microphotograph showing cytological characteristics of the infiltrating embryonic spindle cells. Heart muscle fibers are seen at the right below. Hematoxylin-eosin stain.  $\times 300$ .
- FIG. 8. Case 2. Microphotograph showing invasion of myocardium by tumor tissue. Hematoxylin-eosin stain.  $\times 175$ .



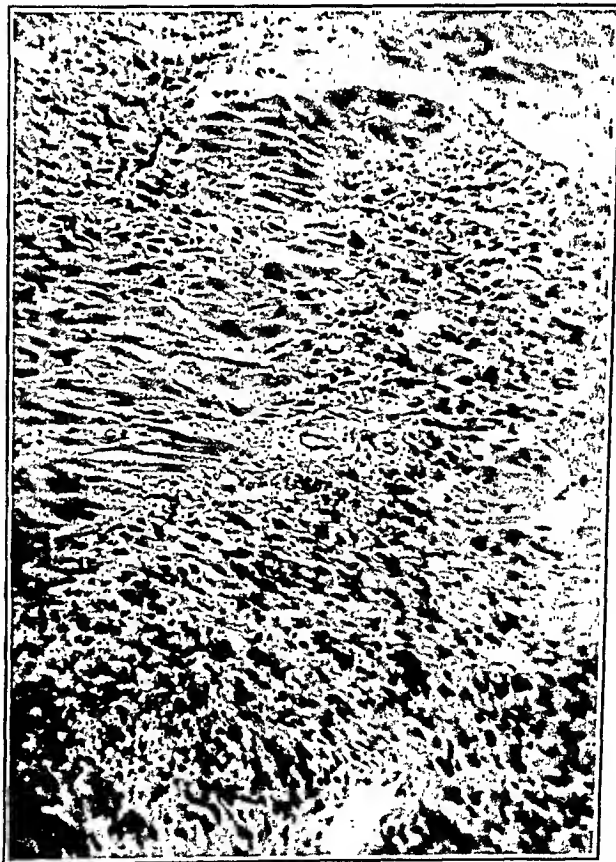
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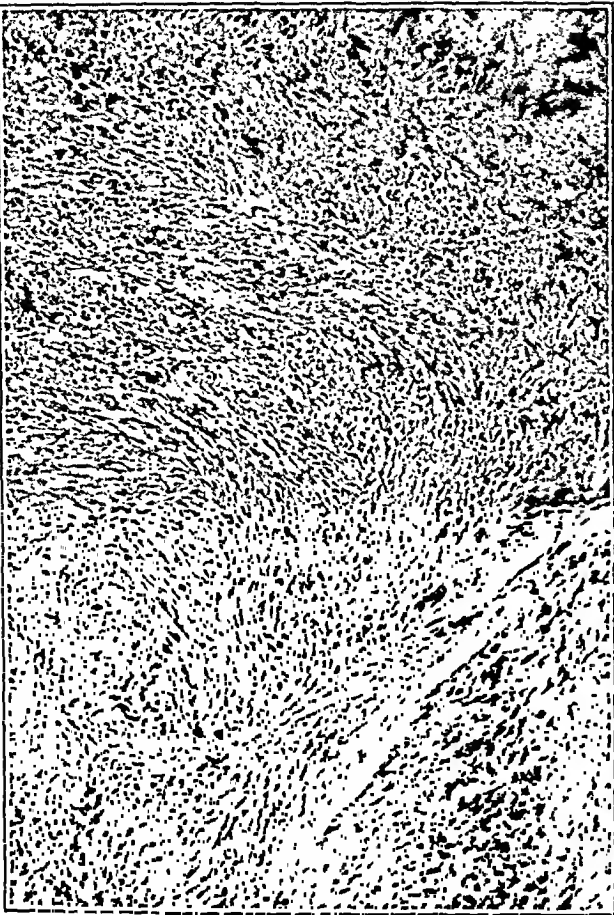
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Choisser and Ramsey

Angioreticoendothelioma of the Heart

PLATE 32

- FIG. 9. Case 2. Microphotograph of an area in which the spindle cell component of the tumor has a "windblown" appearance suggestive of neurogenic origin. Hematoxylin-eosin stain.  $\times 150$ .
- FIG. 10. Case 1. Microphotograph of a section stained by the Bielschowsky method. There has been massive proliferation of fine, compact, lace-like reticulum.  $\times 300$ .
- FIG. 11. Case 2. Microphotograph showing "broken twig" type of reticulum proliferation. Bielschowsky's silver impregnation method.  $\times 300$ .
- FIG. 12. Case 1. Microphotograph showing structure of the reticulum in a vascular portion of the tumor. Each vascular space is enveloped by reticulum which clearly outlines its wall. Bielschowsky's silver impregnation method.  $\times 400$ .



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Choisser and Ramsey



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Angioreticoendothelioma of the Heart



# THE PATHOLOGY OF NUTRITIONAL MUSCULAR DYSTROPHY IN YOUNG RATS \*

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The young of female rats fed certain restricted vitamin E diets develop nutritional muscular dystrophy. The paralytic symptoms of this disease were observed for the first time in the laboratory of Evans and Burr,<sup>1</sup> and the pathology was described by Olcott.<sup>2</sup> Symptoms of weakness of the muscles have been reported in older animals maintained on vitamin E-low diets from the time of weaning (Ringsted,<sup>3</sup> Burr, Brown and Moseley,<sup>4</sup> Einarson and Ringsted,<sup>5</sup> Evans, Emerson and Telford,<sup>6</sup> and Knowlton and Hines<sup>7</sup>). Several of these authors have also described early lesions in the muscles.

The disorder is prevented by the administration of wheat germ oil or vitamin E concentrates to either the mother or her young during the period of lactation (Evans and Burr,<sup>1</sup> Ringsted,<sup>3</sup> Morelle,<sup>8</sup> Mason,<sup>9</sup> and Olcott and Mattill<sup>10</sup>). However, when natural food diets in which the antisterility factor has been inactivated by treatment with ethereal ferric chloride (Waddell and Steenbock<sup>11</sup>) are fed, the young rats remain normal (Goettsch and Pappenheimer<sup>12</sup>). This discrepancy has been substantiated recently by the report of Goettsch and Ritzmann<sup>13</sup> that young rats are protected against disease of the muscles by supplementing the diet with wheat germ oil, with oil of treated wheat germ in which the antisterility vitamin has been inactivated by ethereal ferric chloride, or with  $\alpha$ -tocopherol (vitamin E).

This paper concerns the pathological changes in the young rats studied by Goettsch and Ritzmann.<sup>13</sup> Our observations confirm those of Olcott<sup>2</sup> in all essentials. The alterations are strictly limited to the voluntary muscles. Although the central nervous system and peripheral nerves were not studied in every animal, a searching examination was made in several typically affected

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rats.\* No lesions were found in the brain, spinal cord or peripheral nerves, and even the terminal neurites and end plates were well preserved in the midst of the degenerated muscle fibers (Fig. 1). These findings are in accord with those of Barrie<sup>14</sup> and of Olcott,<sup>2</sup> who found no abnormalities in the nervous system, but are opposed to those of Lipschutz,<sup>15</sup> who described degeneration of crossed and homolateral vestibular tracts, and of the posterior tracts of Goll and Burdach, the median lemniscus, and the tecto-spinal tract. Neither Barrie nor Lipschutz appears to have studied the skeletal muscles.

We have also been unable to confirm the observations of Barrie on the cytological changes in the anterior lobe of the pituitary. In the glands studied by us we failed to note the degranulation of eosinophils and the spongy appearance of the basophils, which this author described as characteristic. The thyroid also showed no striking difference from that of normal rats, an observation in accord with that of Telford, Emerson and Evans.<sup>16</sup> The thymus was well preserved but in one animal showed a moderate accidental involution, which can probably be ascribed to starvation rather than to a specific effect of the diet. The remaining glands of internal secretion, as well as the principal viscera, showed no significant alterations. The myocardium, the smooth muscle of the intestine and other tissues were not affected.

The gross appearance of the muscles varied according to the number of days elapsing after the onset of symptoms. In rats sacrificed on the day when weakness of the muscles first became apparent the muscles appeared moist and somewhat pale. After 2 days the muscles became yellowish white and opaque. After 3 days or more the muscles appeared streaked and gritty. The muscles of both upper and lower extremities, as well as those of the trunk, were involved but not necessarily symmetrically, nor were the individual muscles always affected in their entirety. Often a band of intact non-degenerated fibers persisted on the surface. As in the muscular dystrophy of guinea pigs (Goettsch and Pappenheimer<sup>12</sup>), the muscles of the tongue seemed to be unaffected. The masseter was found to be involved in several cases.

\* We are greatly indebted to Dr. Abner Wolf for his collaboration in the study of the nervous system.

The microscopic changes also depended on the interval between the onset of symptoms and the time that the animals were sacrificed. The earliest change was a necrosis of the fibers, with retraction and separation of the muscle substances into discrete segments and collapse of the intervening sarcolemma (Fig. 2). In some instances the muscle substance underwent complete hyaline necrosis with loss of all fibrillar structure, but at times the longitudinal striations could still be discerned in the necrotic fibers and persisted even after calcium impregnation had occurred.

Accompanying this alteration in the muscle fibers there was noted on the 1st day a marked interstitial edema, in part fibrinous, which led to wide separation of the fibers (Fig. 3). There was also a marked inflammatory reaction, in the early stages of which polymorphonuclear leukocytes predominated. Occasionally these penetrated the necrotic fibers, but in general they remained confined to the interstitial tissue. On the 1st day there was little alteration of the nuclei of the sarcolemma.

In the animals killed on the 2nd day the edema had largely been resorbed and polymorphonuclears had given way to mononuclear histiocytes. The myocytes were becoming activated, their nuclei were vesicular, and their cytoplasm had increased in volume and was basophilic. Mitotic figures were quite numerous (Fig. 4). The segments of necrotic muscle were becoming enveloped in plasmatic multinucleated cell masses, probably derived from the persisting myocytes, although the participation of histiocytes in this process could not be excluded (Fig. 5). In some instances calcification of the necrotic fibers had begun, although this was more conspicuous and was seen more frequently on the 3rd and subsequent days (Fig. 6).

The specimens from the rats killed on the 3rd and 4th days showed in addition marked regenerative activity on the part of the myocytes. Many of them had taken on spindle or cylindrical shapes, and myofibrils were becoming differentiated on their surfaces. With their development into functional muscle cells the staining again became eosinophilic, presumably due to the regeneration of myohemoglobin. In later stages they could still be distinguished from the persistent unaffected fibers by their smaller caliber, by the central location of their nuclei, and by a decrease in myofibrils. In a specimen (Rat 21 c) obtained on

the 3rd day (Fig. 7) the necrotic fibers had been completely resorbed and their place was taken by well differentiated young fibers aligned in the original axis of the muscle.

We have not as yet examined rats at a later stage of recovery but it seems probable that the regeneration is complete in time and that no permanent scarring of the muscle persists.

### DISCUSSION AND CONCLUSIONS

Our material does not permit any positive conclusions as to the pathogenesis of these lesions. One may conceive of such changes as brought about (*a*) by excessive contraction of fibers with segmental ruptures and subsequent necrosis, (*b*) by a direct and selective toxic action on the muscle cells, and (*c*) by angiospastic occlusion, causing anoxemia and infarction. Although speculation is hardly warranted, the fact that the superficial fibers of a muscle, which perhaps receive a more direct and abundant blood supply from the intermuscular fascia, often escape necrosis (Fig. 8) may perhaps be taken as a point in favor of the last hypothesis. We made a careful examination for capillary thrombi in the early stages of the disease but they were not found. However, the capillaries of the muscles are strikingly empty and collapsed when contrasted with the normal muscle tissue.

Whatever the pathogenesis, the degeneration of the fibers seems to occur with almost explosive suddenness. Later stages are those of reaction to the necrotic tissue and of early and active regeneration. It is thus possible to estimate the duration of the symptoms with reasonable accuracy and to predict that survival will be accompanied by practically complete restoration to normal structure.

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## DESCRIPTION OF PLATES

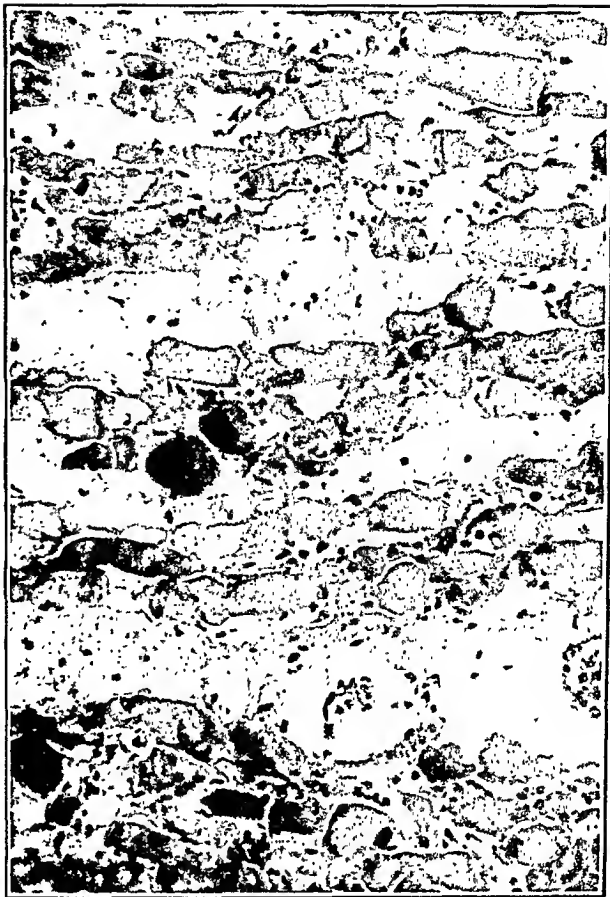
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### PLATE 33

- FIG. 1. Rat G 15. Section showing the preservation of neurites and motor end plates in degenerated muscle. Modification of Cajal's method.  $\times 700$ .
- FIG. 2. Rat G 51 a. Killed on day after appearance of symptoms. Segmental necrosis of muscle fibers, interstitial edema and leukocytic reaction is present. Hematoxylin-eosin stain.  $\times 170$ .
- FIG. 3. Rat G 51 a. Showing a higher power of the same section.  $\times 700$ .
- FIG. 4. Rat G 21 e. Killed on the 25th day, 3 days after the appearance of symptoms. Marked proliferation of myocytes with numerous mitoses is seen. Hematoxylin-eosin stain.  $\times 700$ .



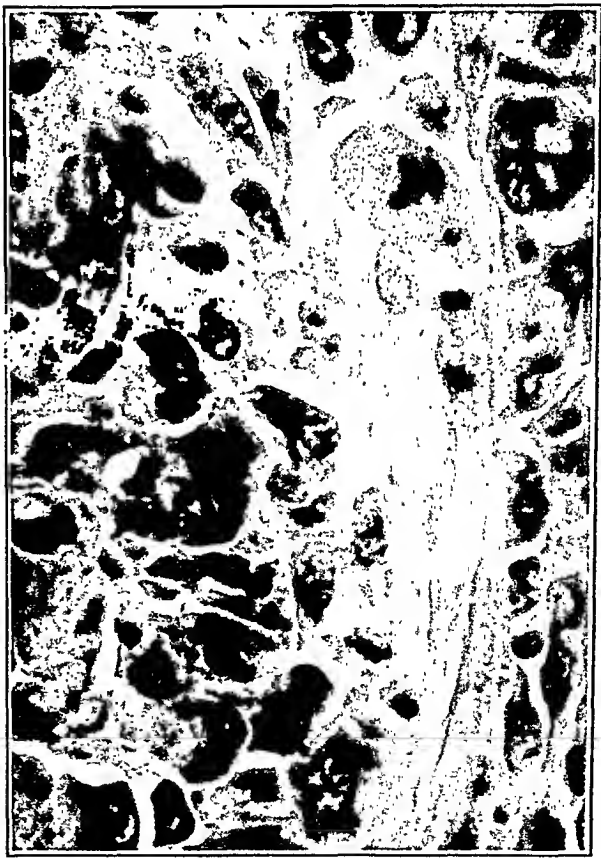
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Pappenheimer

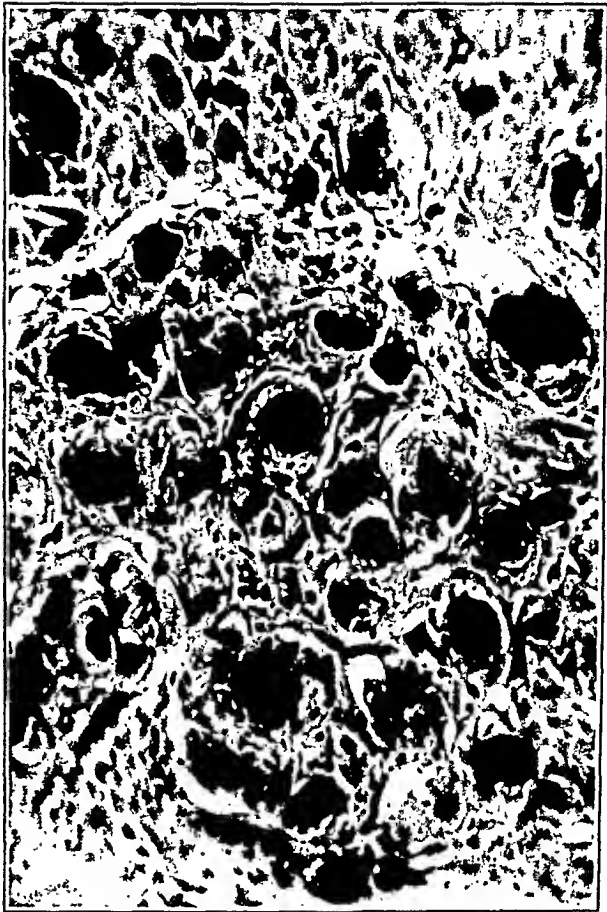
Nutritional Muscular Dystrophy in Rats

PLATE 34

- FIG. 5. Rat G 60 b. Killed on 25th day, 3 days after symptoms were first noted. Necrotic, partly calcified muscle substance surrounded by multinucleated plasmatic masses is seen. Hematoxylin-eosin stain.  $\times 700$ .
- FIG. 6. Rat G 21 d. Killed on 25th day, 3 days after appearance of symptoms. Extensive calcification of necrotic fibers is present. Von Kossa's stain.  $\times 170$ .
- FIG. 7. Rat G 21 c. Killed on 25th day, 3 days after appearance of symptoms. Regenerated fibers of small caliber and centrally disposed nuclei, contrasting with normal unaffected fibers, is seen. Late stage in regeneration. Hematoxylin-eosin stain.  $\times 170$ .
- FIG. 8. Rat G 21 e. Preservation of superficial portion of muscle with advanced degeneration of the central portion is seen. Hematoxylin-eosin stain.  $\times 170$ .



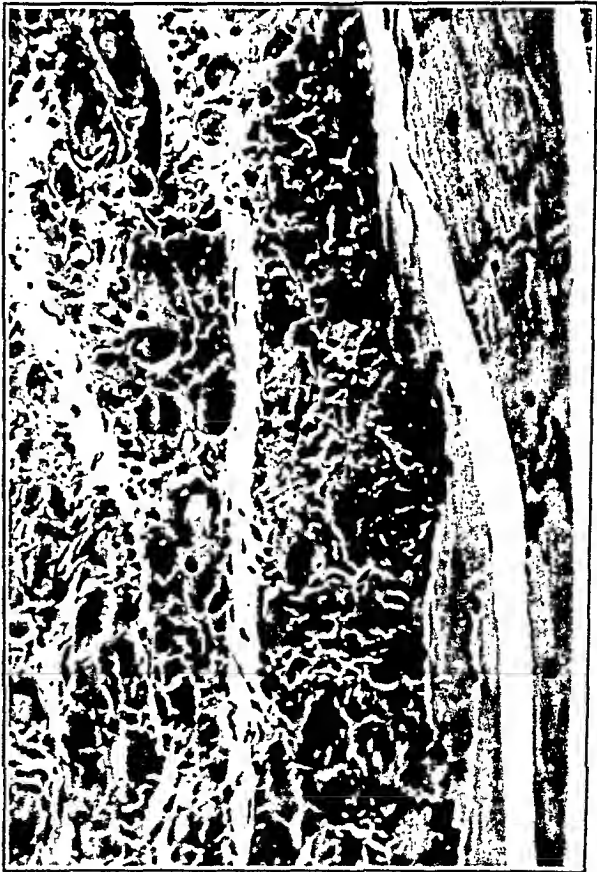
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# THE INFLUENCE OF INFLAMMATION ON THE SKIN NECROTIZING ACTION OF STAPHYLOCOCCUS TOXIN \*

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Although it has been established that under certain conditions an inflammatory reaction may function in producing a non-specific local cutaneous immunity, the basic mechanism involved in such immunity continues to be a subject of opposing hypotheses and divergent opinions. Much has been written concerning the fixation or localization of various irritants in foci of inflammation and the protection afforded the organism as a whole through this reaction. The opposite idea, however, of local protection due to an accelerated removal or dissemination of the irritant has not been adequately investigated.

The findings presented in this communication demonstrate the capacity of a mild cutaneous inflammation to confer protection against the injurious local effects of the toxin of *Staphylococcus aureus*.

Previous studies on the role of inflammation in immunity have dealt with the fate of such substances as dyes, bacteria and foreign proteins following their injection into inflamed tissues.

Menkin<sup>1</sup> demonstrated that when trypan blue was injected directly into an area of inflammation prepared by the subcutaneous application of aleuronat, the dye was not found in either the efferent lymphatics or the regional lymph nodes. The author explains this on the basis of thrombosis of lymphatic vessels and a rapid "walling-off" of the inflamed area. Miller<sup>2</sup> found that inflammation inhibited the absorption of slowly diffusible compounds and increased the dissemination of highly diffusible substances.

The results obtained by Okuneff,<sup>3</sup> on the other hand, indicate that local inflammation may facilitate the dissemination of a colloidal dye. He showed that if a small amount of turpentine was injected into the subcutaneous tissues immediately preceding the injection of trypan blue, a marked acceleration in the rate of absorption of the dye into the blood stream occurred. If, however,

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the dye was introduced 12 hours after the turpentine the rate of absorption of the dye was greatly reduced.

Hudack and McMaster<sup>4</sup> injected dyes into skin sites inflamed by heat, ultraviolet light or bacterial toxins and noted that dye dissemination, as judged by skin decolorization, was greatly accelerated in the inflamed areas. Here the inflammatory reactions were induced 24 hours before instillation of the test dye solution and yet there was no indication of any process of "fixation" or lymphatic thrombosis.

The findings given by Falchetti and Carlinfanti<sup>5</sup> concerning the increased sensitization to anaphylaxis following injection of a foreign protein into a focus of inflammation are probably to be explained on the basis of greater absorption of the antigen from an inflammatory reaction site.

The inadequacy of allergic inflammation as a localizing mechanism is clearly shown in the work of Cannon and Hartley.<sup>6</sup> When they introduced virulent pneumococci directly into sites giving the Arthus reaction the organisms were not localized at the point of inoculation. Animals so treated succumbed to the infection just as quickly as did those in which the microorganisms were injected into normal skin. In some cases the inflammation seemed to favor the growth and dissemination of the bacteria.

#### MATERIALS AND METHODS

The toxin was prepared from stock cultures of *Staphylococcus aureus*, several different strains being employed. Proteose-peptone semisolid medium was dispensed in 100 cc. amounts into 500 cc. Ehrlenmeyer flasks and autoclaved at 15 pounds for 15 minutes. The flasks were inoculated with 8 hour broth cultures, placed in a desiccator with an atmosphere of 20 per cent carbon dioxide and 80 per cent air and incubated at 37° C. for 3 to 4 days. The culture was then filtered through paper, adjusted to a pH of 6.6 and further cleared by centrifugation. The toxic filtrate was preserved by the addition of merthiolate to a concentration of 1:10,000 and was then stored in the refrigerator.

Potent immune serum against sheep serum and against crystalline egg albumin was obtained by injecting rabbits with multiple doses of the corresponding antigen. Injections were continued until the antiserum reached a titer of 1:20,000 to 1:30,000 when

tested against the homologous antigen by the precipitation method.

Healthy young rabbits weighing from 1800 to 2200 gm. were used in these experiments. They were clipped and shaved 2 days before the test and no animals were used a second time.

#### THE INFLUENCE OF ALLERGIC INFLAMMATION ON THE NECROTIZING ACTION OF STAPHYLOCOCCUS TOXIN

In order to determine the effect of allergic inflammation on the ability of the toxin to produce local necrosis of skin, the following procedure was employed.

Fresh, undiluted immune rabbit serum was infiltrated into the prepared skin of a normal rabbit for the purpose of local passive sensitization. Eight to 10 intracutaneous injections of 0.1 cc. each were made in an area of skin about 25 mm. in diameter. Five to 7 hours later the animal was given, intravenously, a dose of antigen specific for the immune serum used, and 0.1 cc. of diluted *Staphylococcus* toxin was injected into the skin of the infiltrated area. Suitable controls were set up for each of the reagents used and daily observations were made as to the character and size of the resulting lesions.

For the sake of convenience the tests were run in three series of 10 animals each. In 6 animals of each series antiegg albumin serum was injected into one flank and antisheep serum in the opposite flank as a further check on the inhibiting effect of the immune serum alone. Table I is a typical protocol showing the results obtained in one such series.

Inasmuch as the area of skin necrosis had usually reached its limit by the end of 48 hours, the dimensions of the necrotic lesion and the number of days required for healing are taken as important points of contrast. As may be seen from Table I, these two observations alone would serve to demonstrate that a mild inflammatory reaction may function to protect a local area of skin against the effects of the toxin. However, the differences in the *character* of the lesions are equally important. The necrotic lesions in the tissues which were the site of allergic inflammation were not only smaller but were also much less severe than lesions in the control areas. In the inflamed areas the necrosis involved only the superficial layers of skin and the brown necrotic crusts that formed after several days were quite thin, while in the control

sites the necrosis often extended to the subcutaneous fascia and the crusts were thick and "leathery."

### THE PROTECTIVE ACTION OF SERUM ALONE

In all of the experiments discussed in the previous section fresh serums, taken less than an hour before use, were employed

TABLE I

*Effect of Allergic Inflammation on Skin Lesions Produced by Staphylococcus Toxin*

Animal No.	Intradermal injections	Interval	Intravenous antigen	Toxin	Size of lesion *	Lesion healed
	<i>serum</i>	<i>hrs.</i>		<i>cc.</i>	<i>mm.</i>	<i>days</i>
17	Right: none Left: antiegg albumin	6	Egg albumin	0.1 0.1	20 × 50 5 × 8	33 14
57	Right: normal rabbit Left: antiegg albumin	5	Egg albumin	0.1 0.1	25 × 35 4 × 7	30 12
26	Right: none Left: antiegg albumin	5	None	0.1 0.1	30 × 30 25 × 35	32 30
56	Right: none (saline) Left: antisheep	7	Sheep serum	0.1 0.1	20 × 40 8 × 8	31 15
70	Right: antiegg albumin Left: antisheep	6	Sheep serum	0.1 0.1	20 × 30 5 × 5	33 13
86	Right: antiegg albumin Left: antisheep	6	Sheep serum	0.1 0.1	30 × 35 5 × 8	34 12
76	Right: antisheep Left: antiegg albumin	5	Egg albumin	0.1 0.1	25 × 40 9 × 9	29 15
66	Right: antisheep Left: antiegg albumin	7	Egg albumin	0.1 0.1	20 × 30 5 × 5	30 14
85	Right: antisheep Left: antiegg albumin	6	Egg albumin	0.1 0.1	30 × 40 8 × 8	32 15
36	Right: antisheep Left: antiegg albumin	6	Egg albumin	0.1 0.1	25 × 35 4 × 9	31 13

\* Taken 48 hours after injection of toxin.

for infiltration of skin areas. Such serums alone rarely caused any inhibition of the toxic action.

It was noted, however, in the course of this work that injections of old serums that had stood in the refrigerator for several days or more would confer protection against the toxin without antigenic participation. Within 5 or 6 hours after cutaneous infiltration with these old serums the site was erythematous and somewhat indurated, and lesions resulting from the injection of *Staphylococcus* toxin into these areas were no more extensive than those in the sites of allergic inflammation. Passage through a Berkefeld filter did not remove the irritating property from these serums; the filtered serums alone would still confer protection against the morbid effects of the toxin.

### DISCUSSION

It is quite evident from these experimental procedures that *Staphylococcus* toxin fails to exert the full force of its typical necrotizing property in the presence of a mild inflammatory reaction. Complete protection is not afforded by this means but marked inhibition of the toxic action is observed with striking regularity.

An important fact with regard to those experiments in which the meeting of antigen and antibody within the tissues was employed as the inflammatory irritant is that the toxin was actually introduced before inflammation was accomplished. Allergic inflammation did not develop, of course, until sometime after the intravenous administration of the homologous antigen. In those instances of infiltration of the skin with aged serum, a mild inflammatory reaction of some 5 hours duration was present at the time the toxin was injected. In either case, however, there resulted a striking diminution in the display of toxic action.

A further point to be considered is that the injection of *Staphylococcus* toxin into the skin is not comparable to the instillation of dyes or foreign proteins or even of virulent staphylococci. We have, in the case of the toxic filtrate, a substance which in itself is capable of setting up severe inflammation with necrosis of tissue. In spite of this the inflammatory and necrotizing potencies of the toxin are greatly lessened under the conditions of the present investigation. Instead of there being an additive action

of the two processes or of the irritating properties of the toxin being superimposed on the allergic inflammation, we observe a retardation or partial loss of the inflammatory propensities of the toxin.

In considering the protective mechanism responsible for the phenomena observed in the present study, the concept of fixation or localization<sup>7</sup> of the toxin does not appear to be tenable here. There is, of course, spreading of the toxin following its injection into normal skin, whereas when this irritant is introduced into inflamed cutaneous tissue the resulting necrosis is confined to a small area immediately around the point of injection. However, if the total amount of the injected toxin is merely fixed or localized in a smaller area, one would expect to see a more intense or more severe reaction than where the whole effect is spread over a larger area. The lesion in the inflamed skin is not only confined to a smaller area, but it is actually less severe and usually heals in half the time of the large lesions.

Likewise, due to the character of the lesions, any explanation based on the idea of inflammation causing a reduction in tissue permeability<sup>8</sup> or inhibition of the spreading factor found in certain toxic filtrates<sup>9</sup> would not seem to be applicable here.

It is a well established fact that in inflammation there are certain changes in the endothelial lining of the blood capillaries which increase their permeability or capacity for passing fluids. One finds in the work of McMaster and Hudack<sup>10</sup> and Hudack and McMaster<sup>4</sup> evidence that the permeability of the lymphatic capillaries is also altered so that they become more permeable under the influence of inflammation. Other workers<sup>11,12</sup> have determined that there is actually an increase in the flow of lymph from a region of inflammation. Drinker and Field<sup>12</sup> conclude that "all evidence points to the fact that the more permeable the lymphatic capillary the better it must function in tissue fluid drainage."

In view of the fact that there is an increase in capillary permeability and a greater turnover of fluid in inflamed tissues, it would appear that the findings of the present investigation could be adequately accounted for on the basis of dilution and rapid removal of the irritant. The mechanism of the protective effect of inflammation under the conditions of the present study is prob-

ably a phenomenon of accelerated dissemination of the toxin from the site of injection into the systemic circulation.

It is not to be inferred from this discussion that the function of inflammation with reference to immunity is similar under different conditions. The character and intensity of the inflammatory reaction and the nature of the irritant must all be taken into consideration in evaluating the protective role. In severe inflammatory processes microorganisms are localized<sup>13</sup> and the vital organs may be protected at the expense of local injury. But, on the other hand, the diffusion of obnoxious substances from a focus of relatively mild inflammation may be facilitated and thus the organism as a whole be threatened.

### SUMMARY AND CONCLUSIONS

Rabbits were used to study the effect of an early inflammatory process on the characteristic skin necrotizing action of *Staphylococcus* toxin. Local cutaneous areas were passively sensitized by the intradermal infiltration of immune serum. A few hours later toxin was injected into the infiltrated site and an allergic inflammation was induced by the intravenous administration of homologous antigen. It was demonstrated that the effect of the toxin was markedly inhibited by the inflammatory reaction, and the necrotic lesions were smaller, less severe and healed much more quickly than the controls.

The significance of these observations as regards accelerated dissemination of the toxin has been discussed.

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## DESCRIPTION OF PLATE

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### PLATE 35

- FIGS. 1 and 2. Rabbit 17. Lesions produced by *Staphylococcus* toxin in normal skin (Fig. 1) and in an area previously infiltrated with anti-egg albumin serum (Fig. 2) 48 hours after the injection of 0.1 cc. of toxin into each area and the intravenous injection of crystalline egg albumin.
- FIG. 3. Rabbit 70. Necrotic lesion resulting from the injection of 0.1 cc. of the toxin into skin previously infiltrated with anti-egg albumin serum.
- FIG. 4. Showing the extent of necrosis in the same animal after injection of 0.1 cc. of toxin into an anti-sheep serum infiltrated area and the intravenous administration of sheep serum. The small areas of discoloration around the central lesion are due to the fading allergic reaction. Both photographs were taken 48 hours after the injection of toxin.



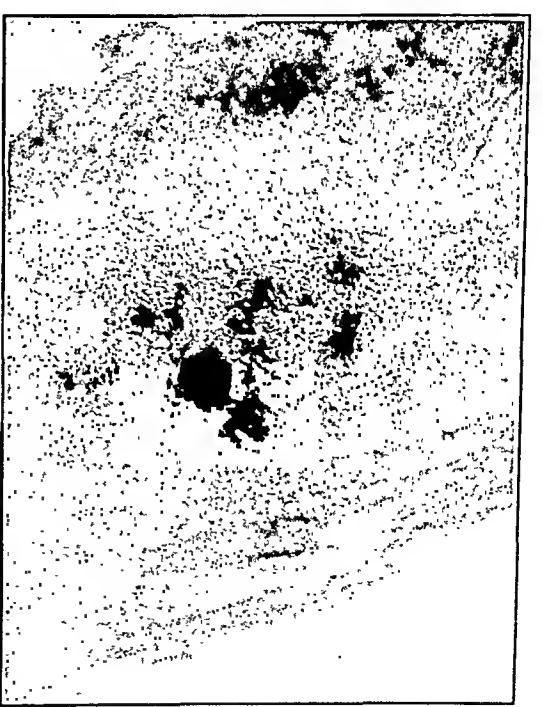
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Kenton

Necrotizing Action of Staphylococcus Toxin



# REPAIR IN EXPERIMENTAL PNEUMOCOCCIC MENINGITIS

## A HISTOPATHOLOGICAL STUDY OF RESIDUAL LESIONS IN RATS \*

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The nature of the residual lesions that follow recovery from purulent meningitis has rarely been described. The only study found in the literature is that by Winkelman and Eckel,<sup>1</sup> which described such lesions in a 10 year old girl who died of an accident 2 weeks after recovery from acute meningococcic meningitis. In this case the pia was reported to be almost entirely free of inflammatory elements, although a relatively slight but general increase in connective tissue was present. The most striking finding was the presence in the cortex of acellular areas (Verödungsherde) of various sizes, most of them small and perivascular. Slight hyalinization and thickening of cortical and subcortical vessels associated with perivascular edema were noted and, at times, pigment-laden phagocytic cells were seen in the Virchow-Robin spaces. A 2nd case with similar findings was mentioned by the same authors.

Although it has been reported that a few experimental animals have recovered from pneumococcic meningitis,<sup>2, 3</sup> no study of residual lesions has been published. Opportunity for such an investigation arose during the course of our various chemotherapeutic experiments on white rats in which pneumococcic meningitis (types I and II) was produced.<sup>4, 5</sup> A significant number of the treated animals recovered and 59 of them were sacrificed 3 to 6 weeks following infection in order to study the brains and spinal cords.

All animals were killed with ether and skinned, the parietal plates removed, and the vertebral column with attached skull was placed in 10 per cent formalin. After fixation the brain and cord were carefully removed so as not to strip the pia. In later experiments considerable time was saved by removing the brain with cord prior to fixation. In most instances the brain was divided

\* Received for publication October 28, 1938.

into five to six coronal sections, and the cord into six to seven cross sections. The tissues were stained with hematoxylin and eosin and by the Nissl method.

Gross examination did not reveal lesions of the brains or cords. Microscopically, however, the pia of every brain and of about one-third of the spinal cords showed pathological changes. The parenchyma of the brain exhibited focal cortical lesions of different varieties in over one-half of the animals.

### MENINGEAL LESIONS

Evidence of a healed or of an active chronic inflammation was present in the leptomeninges of every animal. The lesions, exudative and proliferative in character, were commonly focal, predominantly involved the free surface of the cerebrum and the pons, and were infrequently observed in the cerebral fissures or over the cerebellum. Cellular infiltrations were frequently perivascular, particularly about larger vessels (Fig. 1), but were also found diffusely distributed over limited areas. In addition to lymphocytes and histiocytes there were mononucleated cells with abundant basophilic cytoplasm and central round nuclei, the chromatin of which did not possess the arrangement characteristic of plasma cells. Occasionally, large mononucleated phagocytic cells containing hemosiderin were seen.

A not uncommon change, more or less generalized, was a moderately intense basophilia of the cerebral and cerebellar pia associated with a slight increase in thickness of the membrane and excessive prominence of pial nuclei. A similar prominence of nuclei was observed in many of the smaller meningeal vessels. More frequently, foci of fibrous tissue proliferation were encountered. This connective tissue was usually arranged in a loose meshwork of fibrils throughout which the inflammatory cells were disposed. Occasionally the collagenous fibers formed a thick, wavy, more compact membrane (Fig. 2).

Hemosiderin, usually intracellular, was present in a few foci commonly associated with larger deposits in the superficial cortex. Similarly, calcification of the pia was secondary to larger calcific deposits in the subjacent cortex (Fig. 3).

The meninges of the spinal cords were involved in a manner similar to those of the brain, but less frequently and to a much

lesser degree. The changes were strictly focal and the foci were, as a rule, quite small. The exudation, almost entirely lymphocytic in character, was scanty and the pial fibrosis was slight (Fig. 4). In a number of animals spinal ganglia and nerve roots exhibited peripheral lymphocytic infiltration, and in one instance numerous eosinophils participated. No hemosiderin deposits were encountered although several small foci of calcification were found involving both the pia and the dura.

### BRAIN LESIONS

The brain substance exhibited more striking lesions which, however, were present in only one-half of the animals and were strictly focal and frequently solitary. In some animals extensive shallow cortical scars were found, whereas in others the lesions were deeper and conical in shape with the base toward the surface (Fig. 5). These scars were exceedingly vascular and contained, in addition to many small round cells, larger glial cells and numerous hemosiderin-filled gitter cells. The vessels, most of them of the capillary type, were greatly increased in number and often appeared to spread fan-like from the inferior tip of the wedge shaped scar. They appeared quite prominent, due to the presence of swollen, deeply staining lining endothelial cells and to clusters of perivascular macrophages.

Some of the cortical scars showed calcium deposits in the form of large cohesive masses (Fig. 3), of more loosely dispersed granules (Fig. 6), or of small spicules. In several animals there was a large, subpial ovoid mass of fairly dense collagen in the center of a cortical scar. A concentric arrangement of oval and spindle shaped nuclei contributed a whorl-like appearance to the collagenous mass (Fig. 7). In addition, these masses contained granules of calcium salts and of hemosiderin.

In 2 animals extensive cortical destruction with associated complete disappearance of nerve cells and subsequent scar formation resulted in a marked localized dilatation of the lateral ventricle in that region (Fig. 8).

Cerebellar involvement, consisting of focal atrophy and complete absence of the molecular layer, was found in a few rats. In these regions the pia was slightly thickened and separated from the granular layer only by loosely arranged fibrils, a few lympho-

cytes, and clusters of hemosiderin-filled phagocytes. The granular layer in this region showed a striking absence of Purkinje cells.

In the paraventricular regions many of the animals showed glitter cells containing hemosiderin. This pigment was also present extracellularly.

The focal rarefaction of brain tissue described for the acute stage of purulent meningitis was not found except in and about cortical scars. Such rarefaction with associated disappearance of neurones is well illustrated in Figure 8. At an early stage in this study focal rarefaction of the cerebral cortex was thought to be frequent, but a more careful scrutiny of these foci demonstrated them to be a part of the architectural pattern normal for those particular regions.

### DISCUSSION

The data obtained in the present study indicate that in experimental pneumococcic meningitis resolution of the exudate usually occurs to a degree comparable to that seen in experimental pneumococcic pneumonia of rats.<sup>6,7</sup> Similar to the interstitial inflammation and foci of organization seen following recovery from pneumonia,<sup>6,7</sup> foci of residual inflammation may be found following recovery from pneumococcic meningitis. These foci, however, are relatively few in number and often solitary. It is not unlikely that such solitary lesions might be difficult to find and could easily be missed in the brains of larger animals. Nevertheless, strategically situated in a patient they could conceivably give rise to a variety of nervous manifestations. The increasing incidence of recovery in clinical purulent meningitis<sup>4,8</sup> may afford opportunities for verifying this conjecture.

The lesions herein described were predominantly of a productive inflammatory type and differed thereby from the essentially degenerative lesions described by Winkelman and Eckel.<sup>1</sup>

The proliferation of vascular adventitial macrophages, which played a prominent part in the structure of cortical scars, was similar to the clasmotocytic proliferation described by Kubie<sup>9</sup> in experimental herpetic encephalitis, and later by Stewart<sup>10</sup> in experimental pneumococcic meningitis of dogs.

Finer histological changes, hyalinization with thickening of vessels and perivascular edema were not noted.

## SUMMARY

In a study of the central nervous system of 59 rats that had recovered from pneumococcic meningitis, exudative or proliferative meningeal changes were found in every animal. Lesions of the brain parenchyma, consisting of scars, cellular infiltration and calcium or hemosiderin deposits, were found in about one-half of the rats, while only about one-third exhibited lesions in the meninges of the spinal cord. The spinal meningeal changes were usually slight and were not associated with parenchymatous lesions.

Note: The authors wish to thank Dr. H. T. Karsner and Dr. Harry Goldblatt for their helpful criticism.

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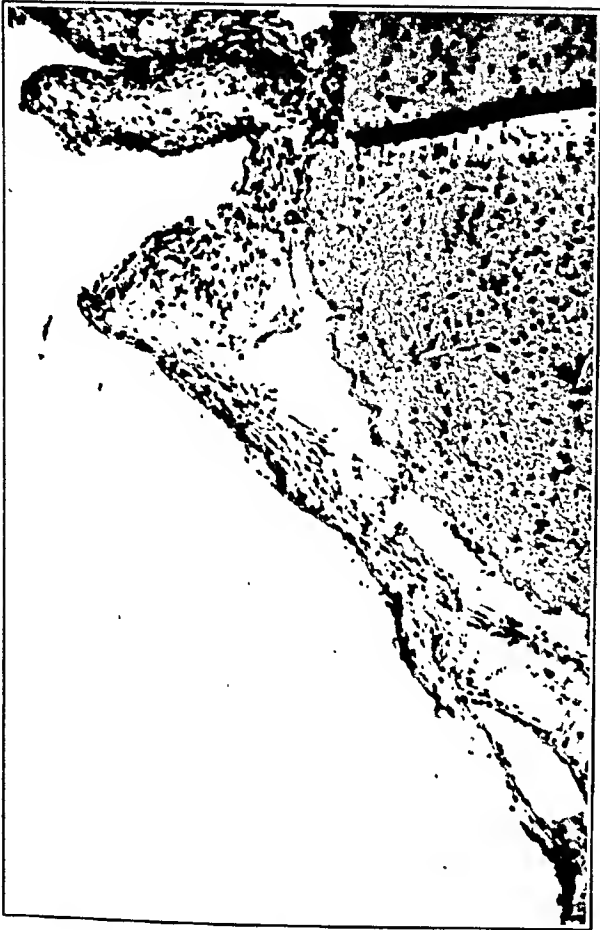


## DESCRIPTION OF PLATES

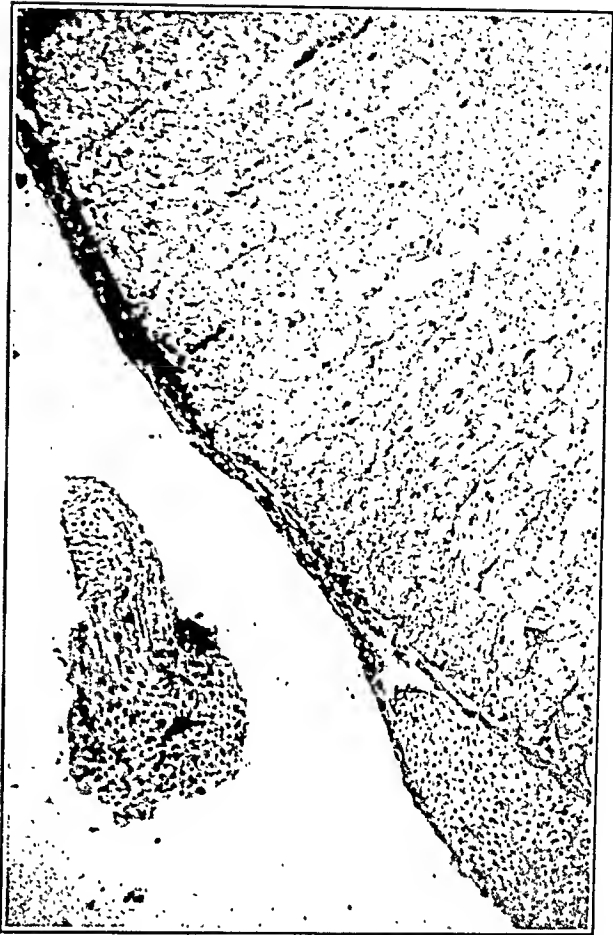
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### PLATE 36

- FIG. 1. Lymphocytic infiltration of the pia arachnoid, predominantly perivascular in character. Type I pneumococcic meningitis. Treatment with sulfanilamide and serum. Rat sacrificed 34 days following infection. Hematoxylin-eosin stain.  $\times 114$ .
- FIG. 2. Fibrosis of the pia arachnoid with associated cellular infiltration. Type II pneumococcic meningitis. Treatment with sulfanilamide. Rat sacrificed 22 days following infection. Hematoxylin-eosin stain.  $\times 100$ .
- FIG. 3. Shallow cortical scar with large cohesive mass of calcific material involving the pia also. Prominent, deeply staining capillaries are seen beneath the mass. An area of rarefaction with clusters of hemosiderin-filled gutter cells is present at a lower level. Type II pneumococcic meningitis. Treatment with sulfanilamide. Rat sacrificed 29 days following infection. Hematoxylin-eosin stain.  $\times 150$ .
- FIG. 4. Lymphocytic infiltration of the spinal meninges. Type I pneumococcic meningitis. Treated with sulfanilamide and serum. Rat sacrificed 34 days following infection. Hematoxylin-eosin stain.  $\times 122$ .



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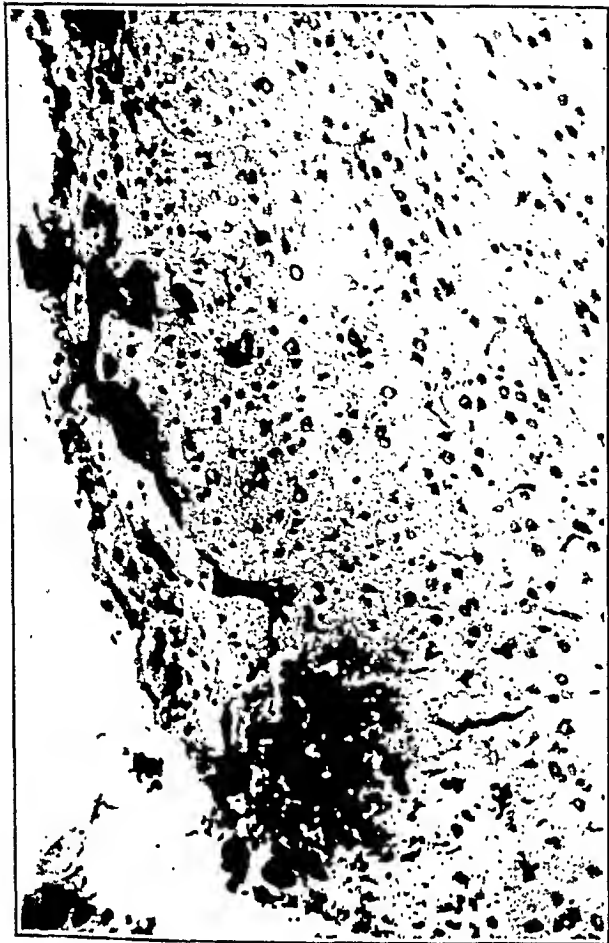
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Gross, Cooper and Lewis

Repair in Pneumococcic Meningitis

PLATE 37

- FIG. 5. Cellular cortical scar containing many hemosiderin-filled phagocytes. Same animal as in Figure 2. Hematoxylin-eosin stain.  $\times 122$ .
- FIG. 6. Cortical scar containing calcium in granular dispersed form. A number of prominent, deeply staining vessels are present. Type I pneumococcic meningitis. Treated with sulfanilamide and serum. Rat sacrificed 38 days following infection. Hematoxylin-eosin stain.  $\times 150$ .
- FIG. 7. Cortical scar containing a large collagenous whorl. The large, deeply staining cells are hemosiderin-filled phagocytes. Same animal as in Figures 2 and 6. Hematoxylin-eosin stain.  $\times 70$ .
- FIG. 8. Extensive shallow cortical scar with rarefaction, absence of neurocytes and atrophy. The concave edge represents the attenuated ependyma of the dilated lateral ventricle. The conspicuous character and large number of the vessels is well illustrated. Type I pneumococcic meningitis. Treatment with sulfanilamide and serum. Rat sacrificed 37 days following infection. Hematoxylin-eosin stain.  $\times 110$ .



6



6



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# THE EFFECT OF DIET ON THE PATHOLOGICAL CHANGES IN RATS WITH NEPHROTOXIC NEPHRITIS \*

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Nephrotoxic nephritis has been regarded by Masugi and the majority of recent workers as the experimental renal lesion which most closely simulates Bright's disease in man.<sup>1,2</sup> Previously we demonstrated that severe acute nephritis induced in rats by means of nephrotoxin generally progressed to a chronic disease with ultimate kidney failure.<sup>2,3</sup> An occasional rat, however, when fed a stock diet, recovered from the acute renal injury, while a number of animals with chronic nephritis of a year's duration still maintained a normal urea clearance value. This variation in the response of individual animals suggested that the course of the disease might be influenced by internal environmental factors, the "milieu intérieur" of Claude Bernard. Since diet has long been considered an important therapeutic measure in the treatment of Bright's disease, and has been shown to affect the clinical picture in one type of experimental renal injury,<sup>4</sup> it seemed desirable to study its influence on the diffuse glomerulonephritis which results from injection of antikidney serum. Accordingly, severe nephrotoxic nephritis was induced in a large group of rats and the course of the renal disease was observed for 10½ months to determine the influence of different diets. Detailed clinical and chemical studies on 48 of the animals in this group are to be reported elsewhere. The present paper deals with the pathological changes found in these rats and in others followed for a shorter period; renal lesions observed in normal rats maintained on one of the experimental diets are also described.

## MATERIALS AND METHODS

*Nephrotoxic Serum:* Antikidney serum was prepared in rabbits by immunization with sterile suspensions of perfused rat kidney. Throughout this study a single serum was used, *viz.* that from

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Rabbit 4557 which was always capable of inducing severe nephritis in rats receiving a total of 0.65 cc. per 100 gm. of body weight, in three divided doses given on consecutive days.<sup>5</sup>

*Animals:* Young hooded rats of the Whelan strain were injected when they weighed about 100 gm. Males and females were distributed equally in the respective diet groups.

*Diet:* Rats were fed on one of three isocaloric diets. Each ration contained 27 per cent fat, 4 per cent Osborne and Mendel salt mixture No. 1,\* and vitamins plus the following constituents: Diet L — 5 per cent protein and 64 per cent carbohydrate; Diet B — 18 per cent protein and 51 per cent carbohydrate; Diet H — 40 per cent protein and 29 per cent carbohydrate. The protein consisted of Lactalbumin, the fat was chiefly Crisco, while the carbohydrate was composed of a mixture of 2 parts Karo powder and 1 part cane sugar. Cod liver oil U.S.P. which made up 5 per cent by weight of each diet contributed part of the dietary fat and vitamins. Brewers yeast 1 gm. (wet) given on alternate days provided further vitamins. Food and water were always available to the rats.

*Technical Procedures:* The methods used for collecting clinical and chemical data have been previously described.<sup>3</sup> Moribund animals were generally etherized in order to obtain well preserved tissues. Organs were fixed in Zenker's solution and in 10 per cent neutral formalin. Paraffin sections were stained with eosin and methylene blue, and in addition, Mallory's aniline blue stain and McGregor's<sup>6</sup> modification of the Mallory-Heidenhain method were applied to sections of all kidneys. The scharlach R method was used on frozen sections to show fatty changes. Other staining techniques were occasionally employed.

## EXPERIMENTAL

### *Clinical Course of Nephrotoxic Nephritis in Rats Maintained on Different Diets*

The course of the experimental nephritis was similar during the first month in all the rats irrespective of the type of diet that was fed.† Severe albuminuria with cylindruria appeared and per-

\* Harris product.

† Data presented in abstract, Farr, L. E., and Smadel, J. E., *Proc. Soc. Exper. Biol. & Med.*, 1937, 36, 472-473.

sisted. Anasarca was present for a variable number of days. Plasma protein values, which were low during the edematous phase, had generally returned to normal 1 month after the injections of nephrotoxin. On the other hand, blood urea nitrogen values and urea clearance determinations remained within normal limits during this early period, except in a few rats which are not included in this report because they succumbed to the acute disease.

The clinical findings in the group of nephritic rats fed Diet L (low protein-high carbohydrate) diverged markedly during the 2nd month from those of the animals fed the other two diets. Seventeen of the 19 animals that received this diet showed either a marked diminution, or, in a few instances, a complete disappearance of urinary abnormalities. Four of these rats were sacrificed at the end of the 3rd month for histological study. Two animals which had excreted urine of a consistently abnormal character died suddenly in the 5th month of the disease without antecedent renal failure. Eight and one-half months after nephritis had been induced, none of the 13 survivors had elevated blood urea or abnormal urea clearance values; moreover, only 1 showed moderate amounts of protein and casts in the urine, 4 had normal urines and the remainder occasionally put forth traces of urinary protein or a few casts. Two of the rats, which had apparently recovered, and 1 of those with slight evidences of nephritis were sacrificed at this time, while 5 of the 10 surviving rats were changed from Diet L to Diet H (high protein-low carbohydrate). During the final 2 months of observation it became apparent that the change to Diet H had had an adverse effect on the diseased kidneys. The single animal in this subgroup with normal urine at 8½ months did not suffer a relapse, but the other rats with low grade or latent nephritis showed an increase in albumin and casts sufficient to warrant a diagnosis of mild or moderate kidney irritation. Exacerbations of this sort did not occur in the rats maintained on the original Diet L.

Every animal fed Diet H (high protein-low carbohydrate) after receiving antikidney serum continued to have marked albuminuria and cylindruria until it died or was sacrificed. Four members of this group were sacrificed 3 months after injection. Only 2 of the 15 remaining rats survived until the experiment was terminated



10½ months after induction of nephritis; moreover, both of these animals were in a terminal phase of chronic progressive nephritis. Among 13 rats dying with renal failure, the average time of survival after injection was 6 months.

The course of the disease in rats fed on Diet B (basal) was similar to that observed in earlier experiments when the animals were maintained on a varied stock laboratory diet.<sup>3</sup> One of the 15 rats in this group recovered clinically during the 2nd month, while 6 showed abnormal urinary contents throughout the experiment but did not develop renal failure. Eight of the 15 rats died of kidney insufficiency; their average time of survival was 5½ months.

Smaller groups of normal rats fed on Diets L and H for several months were sacrificed for histological study. These animals excreted normal urine throughout the period.

#### HISTOPATHOLOGICAL OBSERVATIONS

The characteristic lesions in rats with acute nephrotoxic nephritis and the chronic nephritis that follows the acute injury have been described.<sup>2</sup> Both glomeruli and tubules are affected in the acute disease. The tubular epithelium shows necrobiotic changes, principally hyaline droplet degeneration, while the outstanding glomerular lesion consists of swelling of the glomerular capillary basement membranes.

#### *Renal Lesions in Rats Fed Diet B*

The nephritic animals maintained on this normal basal diet not only followed the same general clinical course as those kept on a stock diet in previous experiments, but also had essentially the same type of kidney damage; hence, only a summary need be given here. Rats that died of progressive nephritis from 68 to 237 days after receiving nephrotoxin had enlarged kidneys with granular surfaces, and many cystic dilated tubules filled with coagulated material were visible macroscopically throughout the cut sections, especially in the corticomedullary region. These changes were most marked in animals with long-standing disease. Microscopic examination showed that practically all the glomeruli were abnormal: those least affected had distorted tufts with irregularly thickened glomerular capillary basement membranes;

in others the epithelium of Bowman's capsule was proliferated and the capsular membrane was thickened, and there were also glomeruli with varying amounts of connective tissue replacement. Glomerular changes of differing degree were always demonstrable in each section, although the number of severely scarred glomeruli was increased in rats with a more chronic disease. Severe damage was found in tubular structures on histological study; extensive dilatation with large hyaline casts, atrophy in areas of interstitial scarring, and epithelial hyperplasia of the remaining functioning units were all observed. The epithelium of the functioning tubules showed various grades of degeneration, including necrosis, in animals killed when moribund. Vascular lesions as well as perivascular and interstitial infiltrations of cells were characteristically present. Rats in this group, which survived the period of the experiment without developing renal failure, even though showing evidence of chronic nephritis until sacrificed, had the same types of kidney lesions, but many functioning renal units remained.

The single animal in this group in which the nephritic process diminished markedly during the 2nd month, and thereafter became negligible, had kidneys with surfaces that were essentially smooth. Moreover, on microscopic examination most of the structural units appeared normal. A rare dilated tubule filled with a hyaline cast, and an occasional small, completely scarred glomerulus could be found. However, fair numbers of glomeruli which appeared capable of functioning, had slight abnormalities in structure. Changes such as irregular thickening of the capillary basement membrane, local dilatation of capillary loops, thickening of capsular basement membrane and, rarely, small crescents were encountered.

The typical macroscopic appearance of a kidney from a rat with progressive nephritis in this diet group is illustrated in Figure 2. Figures 5 and 7 depict characteristic microscopic lesions seen in such kidneys.

### *Renal Lesions in Rats Fed Diet H*

Young normal and nephritic rats thrived when fed the high protein-low carbohydrate diet H; they gained weight faster and grew larger than rats kept on the basal diet B. A normal male

animal weighing 90 to 100 gm. when placed on Diet H usually doubled its weight in  $2\frac{1}{2}$  months. The kidneys of such an un-injected control rat weighed 1.3 to 1.4 gm. each and, except for slight hypertrophy, were normal on inspection and on microscopic examination.

In general, similar kidney lesions were found in rats with chronic progressive nephritis maintained on either Diet B or H; certain differences, however, usually made it possible to distinguish between members of the two groups on macroscopic evidence alone. The kidneys of rats in the Diet H group were usually smaller. Thus, the average weight of the left kidneys of 7 rats dying in Group B was 1.6 gm., while the average of 13 rats dying in Group H was 1.2 gm. The cortical surface was more coarsely granular in Group H. Finally, the cut sections of kidneys from rats in Group B contained many more cystic tubules filled with hyaline material than did kidneys of animals in Group H. These differences were more pronounced in rats which succumbed 5 to 7 months after injection but are clearly depicted in the two kidneys obtained at the end of the experiment and illustrated in Figures 2 and 3.

All gradations of glomerular change described in rats of Group B were observed in animals fed the high protein-low carbohydrate diet; however, the number of completely or extensively scarred glomeruli was always greater in the latter group of animals for any given duration of the nephritis.

A more striking difference between the two groups was found in the varying proportions of the several types of tubular lesions. Cystic tubules filled with hyaline material, observed in such abundance throughout the cortex and medulla of rats with progressive nephritis in Group B, were less frequent and smaller in rats of Group H. On the other hand, necrobiotic changes in the tubular epithelium were more striking in sections from Group H; moreover, degeneration was observed in cells of the proximal segment of tubules in animals of Group H which were killed (3 months after nephritis had been induced) before renal failure was imminent. Widespread interstitial fibrosis encompassing destroyed tubules was marked in the kidneys of the rats in Group H and was observed as early as 3 months after injection of antikidney serum. In addition, hypertrophy and hyperplasia of the epithelium

of remaining functioning tubules were also significantly greater in rats of Group H. Urochrome pigment was conspicuous in the tubular epithelium of rats with terminal anemia irrespective of the diet group. Vascular lesions and perivascular cellular infiltrations occurred with equal frequency and intensity in kidneys of rats that died in the two groups.

In general, glomeruli and tubules which had been damaged by nephrotoxin apparently were unable to undergo repair when the rats were fed the high protein-low carbohydrate diet. Instead, progressive destruction of kidney substance, with connective tissue replacement, proceeded at a more relentless pace than in rats fed the basal diet. Macroscopic and microscopic changes characteristically found in nephritic rats which died while being fed the H Diet are represented in Figures 3, 6 and 8.

#### *Renal Lesions in Rats Fed Diet L*

Diet L was sufficiently well balanced to sustain life and to permit a retarded growth of rats, but various abnormalities were observed in animals maintained on it. For example, both untreated and nephritic rats fed Diet L failed to attain normal weight or stature; their fur continued to be soft and short even after the adult state had been reached; and finally, renal and hepatic lesions were consistently observed. It is necessary to present a description of the characteristic pathological changes found in young normal rats maintained on this diet for several months before attempting to evaluate the lesions attributable to nephrotoxin.

A young female rat (PN-13), weight 66 gm., was fed a stock diet for 2 weeks while repeated urine specimens were obtained; no urinary albumin, blood or casts were demonstrable. The animal was then transferred to Diet L. During the next 2½ months this animal gained from 100 to 130 gm. and continued to excrete normal urine.

The kidney, examined at the end of the period, weighed 0.6 gm. and had scattered shallow depressed scars on the cortical surface. On cut section, the corticomedullary region showed numerous, slightly raised yellow streaks arranged in parallel lines radial to the pelvis (see Fig. 11).

The principal changes found on microscopic examination of this kidney occurred in the areas represented macroscopically by

the yellow streaks. Groups of moderately dilated tubules were lined by low cuboidal epithelium, often so thinned as to resemble the connective tissue cells which lay outside the thickened and hyalinized tubular basement membrane. The lumens of dilated tubules generally contained refractile material, sometimes arranged in concentric rings, which stained very faintly with the acid dyes and failed to stain differentially with scharlach R or iodine. Remnants of a brush border could still be identified in certain of the affected proximal convoluted tubules. In the same area, atrophic tubules lined by epithelium with basophilic cytoplasm and with collapsed lumens were present. Their basement membranes were also hyalinized and surrounded by scar tissue. Less drastic changes were demonstrable in other proximal tubules of the corticomedullary area. In some, only a single epithelial cell was degenerated or necrotic, while in others many were affected and cast off cells lay loose in the lumen. Fat was often demonstrated in such damaged cells by scharlach R stains. Regeneration of epithelium was observed and occasionally resulted in an irregular stratification of cells protruding into the lumen. Thickening of the tubular basement membrane or increase in interstitial connective tissue was not conspicuous here. Figure 12 illustrates the microscopic findings in the corticomedullary region of this kidney.

Most of the glomeruli appeared normal but a few had changes in the tuft and occasionally extensive scarring was observed. Damaged glomeruli were usually found near diseased tubules and occasionally could be shown to connect with one of them. Vascular lesions were not found, and interstitial collections of lymphocytes, when present, were small and limited to the corticomedullary scars.

An extreme fatty change was present throughout the liver. The least affected parenchymatous cells, about the portal spaces, contained fat globules, while in the central portion of the lobules the cytoplasm of individual cells stained a homogeneous red with scharlach R.

The renal and hepatic injury which occurred in all young rats fed Diet L for several months varied in degree. Rat PN-13, described above, was one of the most severely affected of the group. On the other hand, Rat PN-7, which seemed to recover

from nephrotoxic nephritis, had a minimal amount of the kidney damage attributable to the low protein-high carbohydrate diet when sacrificed 3 months after injection of antikidney serum.

A young male rat (PN-7) weighed 55 gm. when nephrotoxin was administered and Diet L was started. The acute nephritis began to subside in several weeks and the urine was normal 4 weeks after nephritis had been induced. The animal weighed 94 gm. when sacrificed 3 months after injection. The kidneys, which weighed 0.5 gm. each, were apparently normal on macroscopic examination but showed glomerular lesions throughout the microscopic section. Practically all of the tuft capillaries contained blood but their walls were significantly thickened by an increased width of the basement membranes. About 20 per cent of the tufts were lobulated or otherwise distorted, and approximately 10 per cent of them were adherent to the capsular epithelium in one or more places; nevertheless, well developed crescents were not seen. A few tufts contained scattered cells, apparently epithelial in origin, with large, bright eosin-staining granules in their cytoplasm. Scattered small areas in the corticomedullary region contained dilated tubules, some of which were filled with laminated pale staining material similar to that seen in Rat PN-13; a few atrophic tubules were also present in these areas. An occasional atrophic tubular structure in the cortex was seemingly related to the pathological areas at the junction of cortex and medulla. In addition, tubules containing hyaline casts were occasionally encountered. Vascular lesions and interstitial cellular infiltrations were not observed.

The liver was the seat of mild fatty change. Microscopic sections of the heart were normal.

The kidneys of rats fed Diet L for 8 to 10½ months after nephritis had been induced with nephrotoxin were similar to those of members of the group sacrificed at 3 months. The lesions attributed to the diet were, however, less obvious at 10½ months. The kidneys of only a few of the animals had macroscopic yellow streaks in the corticomedullary region, which on microscopic examination appeared to be dilated tubules filled with poorly staining material. Narrow bands of old connective tissue arranged radially were present in this portion of all the kidneys; these were often conspicuous but sometimes appeared only once or twice in the

entire section. Five to 10 per cent of the glomeruli in the kidneys from older animals were represented by small contracted scars; in addition a few had moderate hyperplasia of the epithelium of Bowman's capsule. The majority of glomeruli appeared to be functioning but showed abnormalities of the tufts such as described in Rat PN-7. The capillaries of the tufts were often dilated in places and contained puddles of blood; these frequently occurred at the border of the tuft. Scattered atrophic tubules were present throughout the cortex. Vascular lesions and interstitial cellular infiltrations were not present in the kidneys of the group of older rats, nor were generalized vascular changes observed.

The livers of the animals sacrificed at the later date showed about as much fatty change as did those examined earlier; certain of the rats also had cirrhosis.

There was no consistent histological difference detected between the kidneys of rats maintained on Diet L throughout the experiments and those of rats transferred from Diet L to Diet H 8½ months after injection of antikidney serum. Fatty change in the livers of rats in this last group, however, was slight when present.

Two rats, in Diet Group L, failed to make a clinical recovery during the 2nd month and died 134 and 139 days, respectively, after nephritis had been induced. Neither of these animals had a significant reduction of kidney function at any time, but their urine contained 1 to 3 gm. of protein per 100 cc. and numerous casts until the end. Kidney lesions in these 2 rats were more extensive than in other members of the group but hardly severe enough to account for the early death. Changes in the tufts, crescent formation, complete scarring of glomeruli, numerous dilated tubules filled with hyaline casts and degenerative changes in tubular epithelium suggested the picture seen in nephritic rats fed Diet B. Both livers were heavily laden with fat. The death of these 2 animals probably depended on the combined effect of severe chronic nephritis and poor diet.

These histopathological observations agree with clinical data and indicate that rats with severe acute nephrotoxic nephritis tend to recover when maintained on a low protein-high carbohydrate diet. The tubular changes characteristic of the acute nephritis appear to be reversible for the most part when circumstances are propitious. Glomerular lesions, on the contrary, do not disappear;

but notwithstanding the presence of residual changes, most of the glomeruli continue to function.

### *Hematuria in Rats with Chronic and Latent Nephritis*

Pearce<sup>7</sup> held that hematuria was not a result of injury with nephrotoxin but depended on other factors in antikidney serum. We have also emphasized this point in reference to the acute syndrome induced by nephrotoxin.<sup>2</sup> Present observations offer no reason for changing our views on hematuria in the acute nephritis but do indicate that renal bleeding may occur in rats after the acute phase of renal injury has passed.

Hematuria, observed in 7 rats, was generally of the intermittent type and when it occurred was obvious macroscopically. Two rats in Group L showed red cells in the urine for the first time after they had been fed the low protein-high carbohydrate diet for 9 and 10 months, respectively; moreover, there had been neither casts nor more than a trace of albumin for months before the appearance of blood in the urine. Onset of hematuria in the 4 rats of Group B occurred in the 3rd (2 animals), 4th and 7th months and, in a single animal of Group H, hemorrhage was detected during the 7th month on one occasion.

Hematuria in these animals seemed to have no adverse prognostic significance for all of the rats lived throughout the experiment. No evidence of an infectious process which might have accounted for the bleeding was found in any of the kidneys, and cultures of renal tissue made at the time of autopsy were bacteriologically sterile. Dilated capillaries near the margins of diseased glomerular tufts were observed in rats of all three diet groups, and rupture of such diseased capillaries seems a likely explanation for the intermittent hematuria.

### *Generalized Vascular Lesions in Rats with Chronic Progressive Nephritis*

Thickening and hyalinization of the walls of small arteries and of arterioles occurring in various organs of rats which succumbed to chronic progressive nephritis were described and illustrated in an earlier report.<sup>2</sup> Additional vascular changes, such as the presence of fat or of calcium in the media and a reduplication of the internal elastic membrane of coronary arteries, and perivascular



TABLE I  
*Extrarenal Vascular Lesions in Rats that Succumbed to Nephritis*

Diet (protein)	Number of rats	Average time of survival	Heart			Pancreas			Brain			Intestine			Liver			Testicle		
			A	C	T	A	C	T	A	C	T	A	C	T	A	C	T	A	C	T
L (low)	2*	4½ mo.			0/2			0/0			0/2			0/0			0/2			0/0
B (basal)	7	5½ mo.	2/7	6/7	7/7	1/4	4/4	4/4	0/5	4/5	4/5	1/1	0/1	1/1			0/7			0/2
H (high)	13	6 mo.	3/13	7/13	9/13	0/9	2/9	2/9	0/12	2/12	2/12	1/1	0/1	1/1	0/11	6/11	6/11	0/7	3/7	3/7

A = Acute lesions

C = Chronic lesions

T = Total number of rats in each group showing lesions of acute or chronic variety or both

The numerator of each fraction indicates the number of rats with lesions; the denominator the number of organs examined.

\* Rats did not have renal failure immediately before death but had severe chronic nephritis. Death probably resulted from a combination of nephritis and poor diet.

cellular collections in the pancreas were recorded. The occurrence of chronic generalized vascular lesions has also been noted in the present group of rats which died after long-standing nephritis. Their distribution is enumerated in Table I.

Fibrous myocarditis and encephalomalacia which apparently depended on these chronic vascular lesions were found in 13 of 22 rats with chronic nephritis terminating in death; myocardial scarring occurred 5 times in Group B and 3 times in Group H, while encephalomalacia was noted in 3 members of the former group and in 2 of the latter.

In addition to the chronic extrarenal vascular lesions, other changes of a more acute nature were observed, especially in the heart and intestine. These consisted of proliferation and swelling of endothelium, fibrinoid degeneration in the muscle coat, and pyknosis of muscle nuclei. Table I records the frequency of acute vascular lesions such as are illustrated in Figures 13 and 17.

Vessel changes in the heart, brain and pancreas, as well as myocarditis, were found in 1 of the animals of Group B which survived to the end of the experiment; however, its renal function had been depressed for a month before it was sacrificed. Renal insufficiency was present in both rats of Group H when they were sacrificed 10½ months after injection of nephrotoxin. One had vascular lesions in the heart, brain and testicle and also myocarditis, the other had only coronary abnormalities. None of the rats in Group L which were sacrificed during the experiment, or at its end, had generalized vascular lesions.

#### *Acute Myocarditis and Enteritis in Rats with Renal Failure*

An acute focal necrosis of cardiac muscle fibers was frequently seen in rats dying with chronic renal insufficiency. These lesions could usually be identified macroscopically as yellow flecks beneath the epicardium and endocardium. On microscopic examination some groups of degenerated cardiac muscle cells were represented by necrotic debris while others still retained their general outline. The cellular reaction in and about such areas was in the majority of cases mononuclear in type but occasionally consisted of polymorphonuclears or, rarely, was entirely absent. In certain areas cellular collections surrounded muscle fibers which retained their striations. Large mononuclear cells were the prin-

of the cardinal signs of the malignant phase of hypertension in dogs. While blood pressure readings were not made in the present experiment, hypertension was previously demonstrated in rats with chronic progressive nephritis of nephrotoxic origin.<sup>3</sup>

### DISCUSSION

The experiments here presented demonstrate that the course of the nephritis in rats which follows injection of antikidney serum is significantly influenced by at least one internal environmental factor, namely, diet. Clinical evidence of renal disease rapidly subsided in almost all young rats placed on a low protein-high carbohydrate diet after severe nephrotoxic nephritis had been

segment was characteristically found when a basal diet was given, and, finally, destruction of the proximal tubule was outstanding in nephritic rats maintained on a high protein-low carbohydrate diet. The studies of Richards and Walker<sup>10</sup> on selective action of different portions of the kidney tubule in frogs and nepturi suggest an approach to an explanation of our observations in rats, but the exact factors responsible for the recovery of the nephron or destruction of its proximal or distal segment are not clear. The response of the glomerular capsular epithelium was similar to that observed in the remainder of the nephron. Obliteration of the capsular space by crescent formation occurred relatively infrequently in rats of Group L notwithstanding the fact that residual changes in the glomerular capillary bed were common. In contrast to this, marked proliferation of the epithelium of Bowman's capsule with connective tissue ingrowth into the glomerular tuft was conspicuous in the kidneys of rats in both Groups B and H.

Medlar and Blatherwick<sup>11</sup> have commented on the similarity of the renal picture in chronic dietary nephritis observed in rats with unilateral nephrectomy which were maintained on rations rich in animal protein, and the terminal phase of nephrotoxic nephritis in our rats fed a mixed stock diet. The increased susceptibility of male rats to dietary nephritis noted by these workers was less clear-cut in the present experiments; nevertheless 5 of the 7 rats in Group B and both of the animals in Group H that survived the experiment were females.

Generalized vascular changes of both acute and chronic varieties and the visceral lesions that stem from them were encountered only in rats which developed chronic progressive nephritis and renal insufficiency. Diet apparently influenced production of the extrarenal disease only indirectly by way of its effect on the injured kidney. Differences in the frequency of vascular changes were observed in Diet groups B and H, but probably were not significant. Animals that lived throughout the experiment were approximately 380 days old when sacrificed. The failure to find chronic vascular disease or fibrous myocarditis in our surviving animals with normal urea clearance values agrees with the experience of Wilens and Sproul.<sup>12,13</sup> These authors observed cardiovascular lesions in more than half the members of a large group of senile rats. However they state regarding the heart

that "almost all of the changes described make their appearance late in the second year of life and do not attain their maximum incidence until well into the third year." Furthermore, they observed few vascular lesions before the 700th day of life. It may well be that the process of aging and the concomitant development of chronic cardiovascular disease are merely accelerated in rats with severe nephritis.

It should be emphasized that rats used in these studies were all of a black and white hooded strain designated as Whelan stock. Experiments to be reported by Swift and Smadel have indicated that rats of the Wistar and Evans strains respond somewhat differently to the effect of nephrotoxin and diet.

### CONCLUSION

In rats of the so-called Whelan strain the chronic nephritis which follows the administration of antikidney serum can be markedly influenced by isocaloric diets containing different proportions of protein and carbohydrate.

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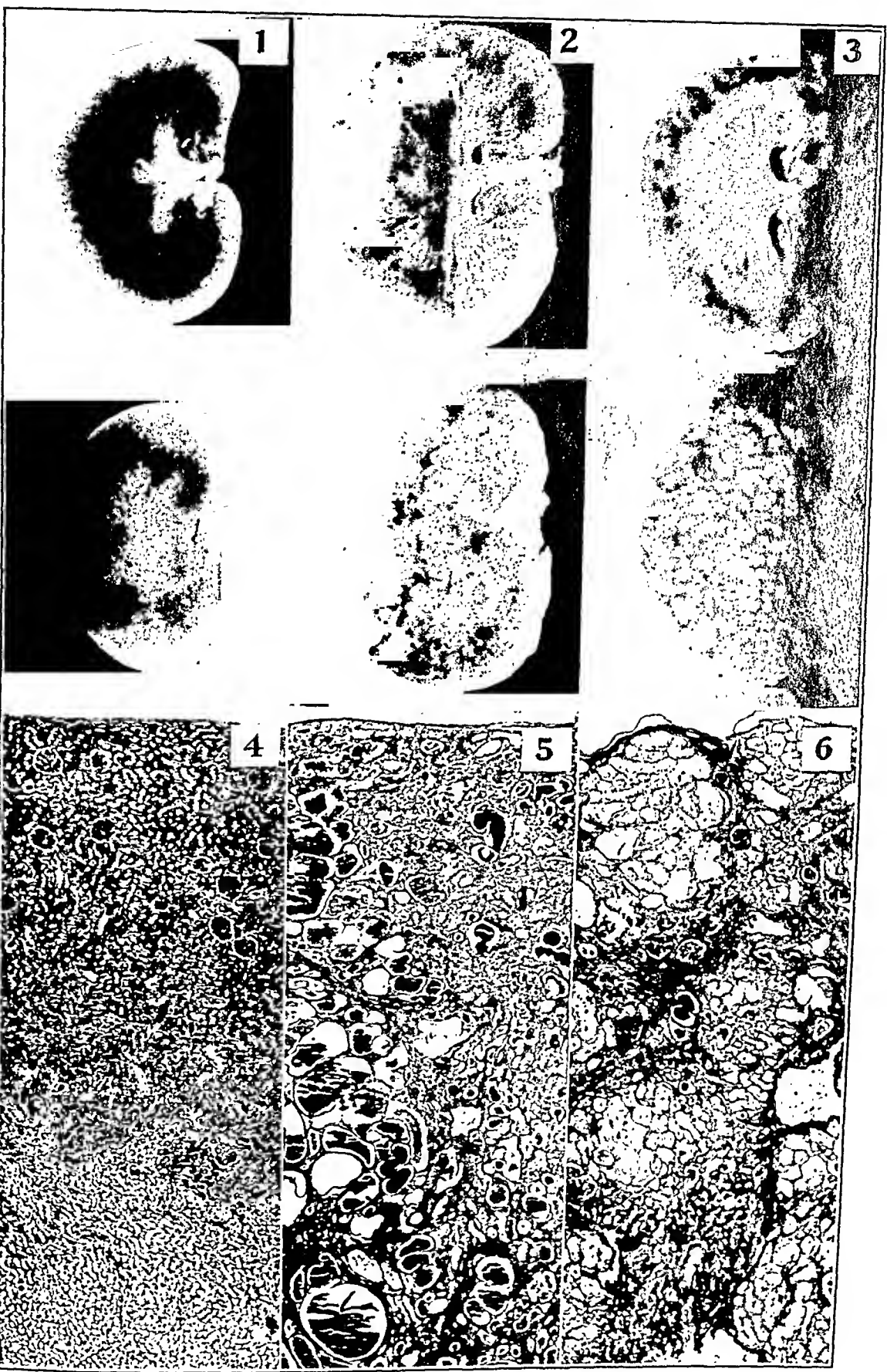
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## DESCRIPTION OF PLATES

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### PLATE 38

- FIG. 1. Cortical and cut surfaces of kidney from Rat 4 L. Rapid recovery from acute nephrotoxic nephritis occurred on the low protein diet; albuminuria had diminished to a faint trace and casts were absent a month after injection of antikidney serum. Autopsied 10½ months after onset of acute nephritis. Left kidney, weight 1 gm.  $\times 3$ .
- FIG. 2. Kidney of Rat 8 B in Diet group B. Severe albuminuria and cylindruria continued from the time of induction of acute nephritis until the rat was sacrificed 10½ months later; final urea clearance value was within the lower limits of normal. Kidney weight 2.1 gm.  $\times 3$ .
- FIG. 3. Kidney of Rat 3 H fed the high protein diet for 10½ months after injection of nephrotoxic serum. Severe nephritis was evident throughout and a depressed urea clearance was observed during the 2 months preceding sacrifice. Kidney weight 1.7 gm.  $\times 3$ .
- FIG. 4. Rat 4 L. Few abnormalities are detectable in the section of kidney at this magnification. Mallory's aniline blue stain.  $\times 35$ .
- FIG. 5. Rat 11 B, basal diet group, died with renal failure 7 months after onset of nephritis. Extensive destruction of tubules and distortion of glomeruli are evident, but the most conspicuous abnormality is the markedly dilated tubules filled with hyaline material. Mallory's aniline blue stain.  $\times 35$ .
- FIG. 6. Rat 5 H, high protein diet, succumbed to kidney insufficiency 5½ months after receiving nephrotoxin. Glomerular damage is similar to that found in Fig. 5. Nests of hypertrophic tubules constitute the bulk of the substance. Extensive areas of interstitial scarring have replaced destroyed tubules. Mallory's aniline blue stain.  $\times 35$ .



Smadel and Farr

Effect of Diet on Rats with Nephritis

PLATE 39

FIGS. 7 and 8. Higher magnification of the same sections illustrated in Figs. 5 and 6 respectively.  $\times 150$ .

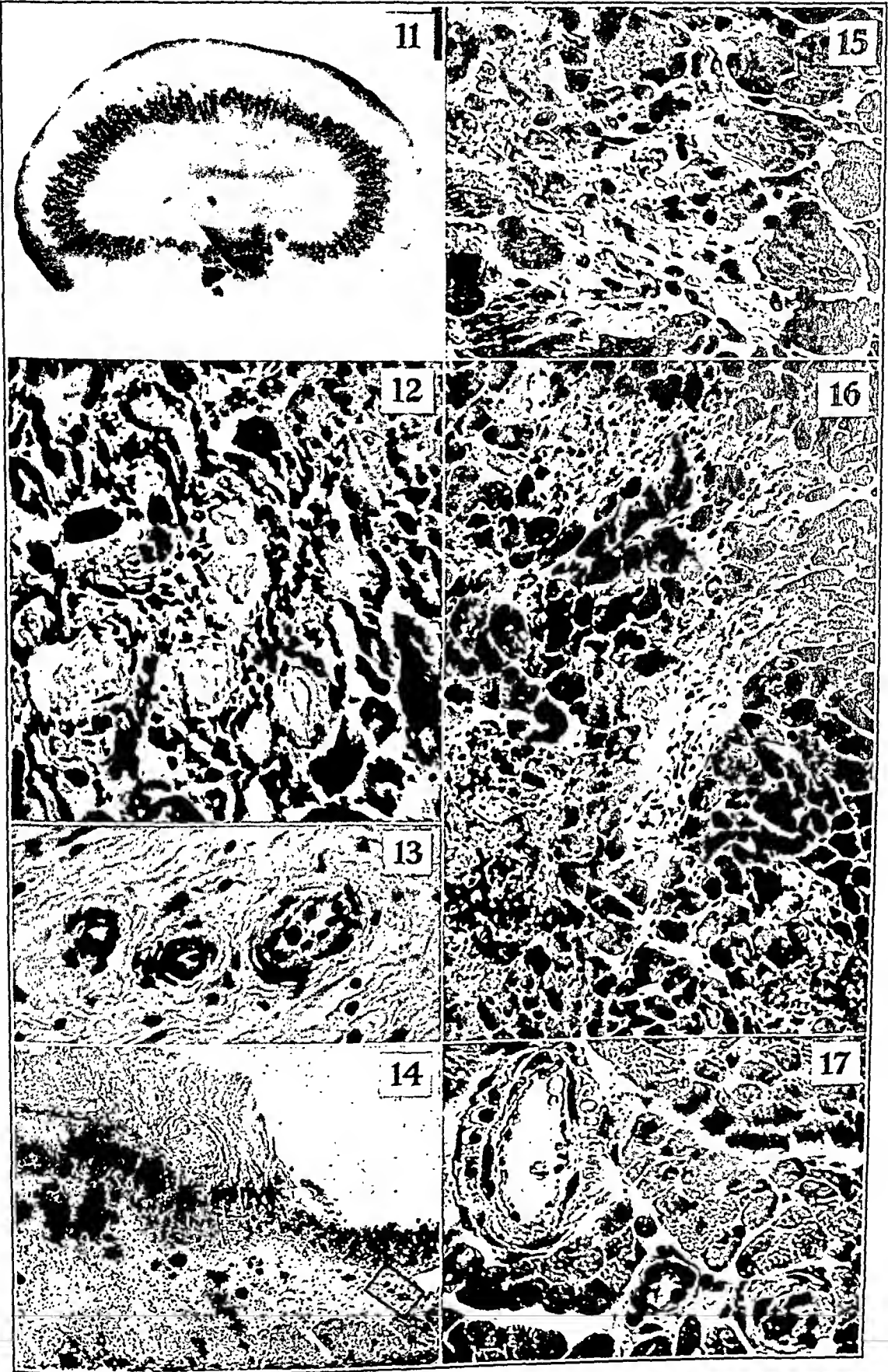
FIG. 9. A higher magnification of the section shown in Fig. 4. The tubules are not significantly altered and the glomeruli appear capable of functioning. Moderate irregular thickening of the glomerular capillary basement membranes is apparent. Some distortion of the glomerular tuft on the right is to be seen, while slight proliferation of the capsular epithelium and a small capsular adhesion can be observed in the glomerulus on the left.  $\times 175$ .

FIG. 10. An area of encephalomalacia in the cortex of the parietal lobe of Rat 13 B, basal diet group, which died of renal failure  $5\frac{1}{2}$  months after nephritis had been induced. Eosin-methylene blue stain.  $\times 70$ .





- FIG. 11. Kidney of Rat PN 13, an uninoculated control animal which was fed the low protein diet for 3 months. The pale streaks in the corticomedullary region were yellow in the fresh specimen.  $\times 4$ .
- FIG. 12. Higher magnification of Fig. 11, showing degenerated tubules with casts in the corticomedullary region. Eosin-methylene blue stain.  $\times 250$ .
- FIG. 13. Section of the cecum of Rat 8 H, which was fed the high protein diet and which died 7 months after the injection of nephrotoxin. An acute necrotizing process is present in all three vessels. These vessels can also be seen at the extreme right in Fig. 14. Eosin-methylene blue stain.  $\times 210$ .
- FIG. 14. Same as Fig. 13. Hemorrhage and edema in the submucosa with a fibrinous exudate covering the necrotic mucosa are shown.  $\times 30$ .
- FIG. 15. Section of the heart of Rat 15 B, basal diet group, which succumbed with nephritis in the 7th month. A small area of focal necrosis of muscle fibers and infiltration with mononuclear cells is seen. Eosin-methylene blue stain.  $\times 450$ .
- FIG. 16. Same as Fig. 15. Multiple focal lesions. An area of fibrinoid change surrounded by muscle fibers showing fresh necrosis is present at the lower left. Areas of necrosis with cellular infiltrations are shown at the lower right and upper portions of the microphotograph. Thickening of vessel walls was also present in other portions of the section. Eosin-methylene blue stain.  $\times 175$ .
- FIG. 17. Heart of Rat 10 B, basal diet group, which died with nephritis after 68 days. Swelling and vacuolation of cells of the media are prominent in the large vessel on the left. Hyperchromatic enlarged cells are seen in the wall of the small vessel in the lower center as well as swelling of the intimal cells. The vessel at the lower right is completely occluded by intimal proliferation. Eosin-methylene blue stain.  $\times 450$ .





# CARCINOMA OF THE PANCREAS \*

## AN ANALYSIS OF FORTY AUTOPSIES

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In a previous communication <sup>1</sup> we analyzed 40 cases of primary carcinoma of the biliary system encountered among 6050 autopsies on persons over 1 year of age performed by the staff of the Department of Pathology of the Charity Hospital of Louisiana, New Orleans, between Jan. 1, 1931, and Oct. 6, 1937. The present study is based on 40 cases of primary carcinoma of the pancreas encountered in the same series of autopsies. This analysis, similar to the previous one, is principally concerned with the site of the primary growth, its structure, and its spread locally and to distant parts. The clinical manifestations and their duration, and the immediate cause of death in the afflicted patients are also touched upon briefly.

*Race, Sex and Age:* Twenty-two of the patients were negro (18 male, 4 female) and 18 were white (17 male, 1 female). One of the patients died in the 3rd, 1 in the 4th, 7 in the 5th, 8 in the 6th, 16 in the 7th, and 7 in the 8th decade of life. The youngest patient was 26 and the oldest 80 years of age (Tables I and II).

*Site and Structure of Neoplasms:* Of the 40 neoplasms, 31 were situated in the head and 9 in the tail of the pancreas. Of the neoplasms primary in the head, 21 were 8 to 10 cm. in diameter or larger. The diameter of the remaining 10 varied from 2 to 6 cm. Of those primary in the tail, 4 were 2 to 5 cm. in diameter, and the remaining 5 were larger.

Whether the growths were primary in the head or in the tail of the pancreas, the gross appearance was practically the same. An infiltrating, firm, gray or yellow-white mass sprinkled with opaque yellow and red or brown areas usually formed the bulk of the growth. At the periphery the mass blended with the pancreatic tissue along a zig-zag line. Sectioning the growth fre-

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TABLE I  
Data on Patients with Carcinoma Primary in the Head of the Pancreas

Number of case	Age yrs.	Sex and race	Spread		Clinical manifestations	Operation	Duration of illness mos.	Cause of death
			Regional	Distant				
H-1 '34-717	26	M C	Lymph nodes	Lymph nodes	Loss of weight, jaundice	None	7	Carcinoma
H-2 '32-312	33	M W	Lymph nodes, peritoneum	Pleurae, heart	Loss of weight, jaundice	Cholecystectomy	4	Carcinoma
H-3 '34-1248	41	F C	None	None	Jaundice	Choledochoduodenostomy	6	Carcinoma
H-4 '36-710	45	M C	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	Laparotomy	4	Carcinoma
H-5 '32-873	47	F C	None	None	Loss of weight, jaundice	Laparotomy	6	Carcinoma
H-6 '33-316	47	F W	Lymph nodes	Liver, lungs	Loss of weight, jaundice	Laparotomy	3	Carcinoma
H-7 '35-690	47	M C	None	None	Loss of weight	Laparotomy	2	Carcinoma
H-8 '35-1110	47	M C	Lymph nodes, peritoneum	Liver, lungs	Loss of weight, jaundice, ascites	None	8	Carcinoma
H-9 '33-1151	49	M C	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	Laparotomy	3	Carcinoma
H-10 '33-845	50	F C	Lymph nodes, duodenum	None	Loss of weight, jaundice	Laparotomy	5	Carcinoma
H-11 '34-940	50	M W	Lymph nodes, duodenum	Liver	Ascites, jaundice, cholelithiasis	None	4	Peritonitis
H-12 '32-248	51	M W	Lymph nodes	Liver	Ascites, jaundice, cholelithiasis	None	24	Carcinoma
H-13 '36-1495	53	M C	Lymph nodes	Liver	Loss of weight	None	4	Diffuse pneumonia
H-14 '36-1028	55	F C	Lymph nodes, peritoneum	Liver	Jaundice, hemoptysis	Laparotomy	14	Pulmonary tuberculosis, hemorrhage
H-15 '34-1302	55	M W	Lymph nodes	None	Jaundice	Cholecystgastrotomy	3	Gastric hemorrhage
H-16 '33-679	61	M W	Lymph nodes, duodenum	Liver	Jaundice, cholecystitis	Cholecystostomy	2	Carcinoma
H-17 '33-566	61	M W	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	Cholecystoduodenostomy, gastroenterostomy	4	Gastric hemorrhage

H-18 '35-739	62	M W	Lymph nodes	Liver	Jaundice	Cholecystenterostomy	7	Carcinoma
H-19 '36-1440	62	M W	Lymph nodes, peritoneum	None	Loss of weight, ascites	None	8	Focal pneumonia
H-20 '32-720	63	M W	Lymph nodes, peritoneum	None	Loss of weight	Cystotomy	8	Prostatic enlargement, pyelonephritis
H-21 '37-498	65	M C	Lymph nodes, peritoneum	Liver	Ascites	None	3	Typhoid fever
H-22 '34-590	66	M C	Lymph nodes	Liver	Loss of weight, jaundice, ascites	None	2	Carcinoma
H-23 '36-465	66	M W	Lymph nodes, peritoneum	None	Loss of weight, jaundice, ascites	None	7	Carcinoma
H-24 '33-1012	66	M W	Lymph nodes	Liver	Loss of weight, ascites	Prostatectomy	6	Tertian malaria
H-25 '32-568	67	M C	Lymph nodes, peritoneum	None	Loss of weight, jaundice	Gastroenterostomy	8	Carcinoma
H-26 '33-574	69	M C	Lymph nodes, duodenum	Liver	Loss of weight, jaundice	None	2	Carcinoma
H-27 '31-292	70	M W	Lymph nodes, peritoneum	Liver, lungs, kidney (left)	Ascites	None	3	Carcinoma, aortic aneurism
H-28 '32-52	70	M C	Lymph nodes, duodenum	Liver	Jaundice	None	2	Carcinoma
H-29 '36-564	70	M C	Lymph nodes	Liver	Loss of weight	None	1	Carcinoma
H-30 '33-775	72	M W	None	None	Jaundice	None	7	Carcinoma
H-31 '35-456	80	M C	Lymph nodes, peritoneum	Liver, pleurae, pericardium	Loss of weight, ascites	None	5	Carcinoma

TABLE II  
Data on Patients with Carcinoma Primary in the Tail of the Pancreas

Number of case	Age yrs.	Sex and race	Spread		Clinical manifestations	Operation	Duration of illness mos.	Cause of death
			Regional	Distant				
T-1 '36-1075	50	M W	Lymph nodes, peritoneum, spleen	Liver, lungs, heart, kidneys, adrenals	Loss of weight	None	5	Carcinoma
T-2 '36-496	51	M C	Lymph nodes, peritoneum	Liver	Loss of weight	Duodeno-jejunostomy	6	Carcinoma
T-3 '32-913	60	M W	Lymph nodes, peritoneum	Liver	Loss of weight, jaundice, ascites	None	8	Carcinoma
T-4 '34-161	62	M C	Lymph nodes, peritoneum, spleen	Liver, adrenals	Loss of weight	Laparotomy	3	Focal pneumonia
T-5 '32-695	65	M C	Lymph nodes, peritoneum	None	Loss of weight	None	6	Carcinoma
T-6 '37-319	66	M W	Lymph nodes, peritoneum	Liver	Jaundice, ascites	None	4	Carcinoma
T-7 '34-1049	67	M C	Lymph nodes, peritoneum	Liver	Loss of weight, ascites	None	7	Carcinoma
T-8 '35-810	75	M C	Lymph nodes, peritoneum	Liver, lungs, pericardium, kidneys	Loss of weight, ascites	None	5	Carcinoma
T-9 '32-44	76	M W	Lymph nodes	Liver, lungs	Loss of weight	None	1	Thrombosis, vena portae



quently elicited a gritty sensation. The cut surfaces disclosed a pattern similar to the external appearance with occasional small cavities containing fluid and necrotic or hemorrhagic débris.

All the growths, whether primary in the head or in the tail, were columnar cell carcinomas. There was, however, some variation in the cellular morphology of the parenchyma and in the amount of stroma. Usually low cuboidal or tall columnar neoplastic cells formed acinar or tubular structures and were embedded in a scant loose or dense fibrous connective tissue stroma. In some instances the neoplastic tissue was distributed amid fairly intact pancreatic tissue; in others it was within masses of connective tissue rich in collagenous bundles containing remains of scattered small acini and dilated ducts. Areas of necrosis and hemorrhage were frequent in the centers of larger tumor masses. In many areas devoid of acini and containing only dilated pancreatic ducts islands of Langerhans were prominent. An occasional island was invaded by neoplastic tissue (Fig. 1). In some of the neoplasms the cells were tall columnar with pale staining cytoplasm and basally located nuclei. In others the cells were low columnar or cuboidal with a granular or basophilic cytoplasm, and mitoses seemed more frequent. In a few instances the tall columnar cells resembled those lining the large ducts, as if the growth had arisen from them (Fig. 2). In none of the growths did the cellular structure resemble the islands of Langerhans.

*Manner of Spread:* Local extension of the growth with involvement of regional lymph nodes occurred in 38 of the 40 cases (95 per cent). Carcinoma primary in the head of the pancreas readily invaded the duodenum (8 instances) and that primary in the tail spread over the peritoneum. Metastases were encountered in the liver in 25 instances, in the lungs and pleurae in 6, in the heart and pericardium in 3, in the kidneys in 3, and in the suprarenal glands and the spleen in 2 instances each.

*Clinical Course:* Obstruction of the common bile duct with varying degrees of jaundice occurred in 22 of the 31 patients with carcinoma in the head of the pancreas, and 10 had ascites. Jaundice occurred in 2 of the 9 patients with carcinoma in the tail of the pancreas, and 4 had ascites. Loss of weight was a prominent feature in practically all instances.

Operations were performed on less than one-half of the patients.

In 38 of the 40 patients the illness lasted from 1 to 8 months, with an average duration of  $4\frac{1}{2}$  months. The newgrowth was the principal lesion and the immediate or contributory cause of death in all but 5 patients.

### COMMENT

The older reports on carcinoma of the pancreas are reviewed by Kiefer,<sup>2</sup> who analyzed 33 cases observed at the Peter Bent Brigham Hospital up to the year 1927. Stout<sup>3</sup> reviewed 33 cases recognized at the Presbyterian Hospital in New York City up to 1932. Leven<sup>4</sup> in 1933 analyzed 99 cases at the University of Minnesota. According to Eusterman and Wilbur,<sup>5</sup> approximately 403 cases of pancreatic carcinoma, verified by operation or autopsy, were seen in the Mayo Clinic during the period between 1921 and 1931. The article by Whipple, Parsons and Mullins<sup>6</sup> in 1935 on the surgical treatment of carcinoma of the ampulla of Vater renewed interest in the subject, and since its publication a number of reports have appeared dealing with the clinical aspects of the problem. The most recent are those by Rives, Romano and Sandifer,<sup>7</sup> Brunschwig,<sup>8</sup> Cooper,<sup>9</sup> Whipple,<sup>10</sup> Ransom,<sup>11</sup> and Crile.<sup>12</sup>

In the reports assembled by Leven the ratio of males and females varied from 3:2 to 4:1. In our own series the proportion was 7:1, although in our total series of autopsies the proportion was 2:1. According to Leven carcinoma of the pancreas occurs most frequently in the 5th, 6th and 7th decades of life. This is true for our series, although over half of our patients were in the 7th decade or older.

The primary growth was located in the head of the pancreas about three times more frequently than in the tail. This proportion approximates the observations of others.

No appreciable difference was noted in the gross or microscopic appearance of the growths arising in the head and in the tail of the pancreas. In a number of instances islands of Langerhans were prominent amid the neoplastic tissue, as if spared by the growth. In a few instances, however, the growth invaded some of the islands. The prominence of the islands amid the neoplastic tissue is mentioned by Ewing<sup>13</sup> and also by Warren.<sup>14</sup>

## SUMMARY

In 6050 autopsies on persons over 1 year of age there were 40 cases of primary carcinoma of the pancreas.

Males and females were represented in the proportion of 7:1. Twenty-three of the patients were over 60 years of age.

The average duration of illness was  $4\frac{1}{2}$  months.

Thirty-one neoplasms were situated in the head and 9 in the tail. All were columnar cell carcinoma.

Carcinoma primary in the head of the pancreas readily invaded the duodenum and that primary in the tail spread over the peritoneum. Metastases were observed in the liver in 25 instances.

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#### DESCRIPTION OF PLATE

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##### PLATE 41

- FIG. 1. H-10. Neoplastic epithelial cells are seen within the island of Langerhans. The cell cords are spread apart.
- FIG. 2. T-2. The neoplastic tall columnar cells with their lightly stained cytoplasm and basally placed nuclei resemble the lining cells of the large ducts, as if the growth had arisen from them.



I



2



# EXPERIMENTS ON THE SOLUBILITY OF HEMOSIDERIN IN ACIDS AND OTHER REAGENTS DURING AND AFTER VARIOUS FIXATIONS \*

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Virchow<sup>1</sup> first in 1847 reported on the solubility of blood pigment in acids, finding that the granular intracellular form was soluble in warm concentrated sulphuric acid, but resisted cold sulphuric acid, alkalies and other reagents. The pigment was reprecipitable from its sulphuric acid solution with an excess of ammonia as brown floccules, which after ashing and solution in hydrochloric acid gave the Prussian blue reaction. Previous treatment of the pigment with alkali made it soluble in nitric acid and more quickly soluble in sulphuric.

Following the introduction of the ferrocyanide reaction by Perls<sup>2</sup> in 1867, and of the ammonium sulphide reaction by Quincke<sup>3</sup> in 1880, little attention was paid to the acid solubility of hemosiderin until the publication of Hueck's monograph in 1912. Neumann<sup>4</sup> in 1888 had noted the pigment showed a quite notable resistance to mineral acids. Hueck<sup>5</sup> stated that hemosiderin may require a very thorough treatment with aqueous solutions of acids, less with alcoholic solutions, to dissolve it, and published a table in which hemosiderin was designated as "soluble in acids" without qualification. This table was reprinted and the statements repeated by Hueck in Krehl and Marchand's *Handbuch der allgemeinen Pathologie* in 1921,<sup>6</sup> and since then the bald statement that hemosiderin is soluble in acids has appeared in various texts on general pathology and on pathological technique (Oberndorfer,<sup>7</sup> Mallory and Wright,<sup>8</sup> Mallory,<sup>9</sup> Schmorl,<sup>10</sup> and von Gierke<sup>11</sup>).

Acid extraction has been used by various workers to remove hemosiderin when studying other pigments. Brown,<sup>12</sup> Seyfarth,<sup>13</sup> and Mayer<sup>14</sup> each used oxalic acid to remove hemosiderin while studying the possible iron content of malaria pigment. Brown and Seyfarth used 12 hours extraction with 2 per cent oxalic acid and

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found iron-reacting pigment afterward. Mayer prolonged this to 18 hours and found the reaction positive in some cases and not in others, but the iron-containing pigment was found only in phagocytes, not in red corpuscles. Glasunow<sup>15</sup> extended the extraction to 24 hours and found no remaining iron. He showed that concentrated oxalic acid did not dissolve malaria pigment in 48 hours and speculated that 12 hours treatment with 2 per cent oxalic acid might be insufficient to remove hemosiderin, but did not try extraction of known hemosiderin. He further noted the

TABLE I

*Hemosiderin Remaining in Formalin-Fixed Paraffin Sections after Varying Exposures to Varying Concentrations of Various Acids*

Stock acid	Dilution	Time	Temperature (room)	Material	Amount of hemosiderin remaining
None	%	hrs.	C.		
Conc. HCl	1	48	30°	S-6896	++++
Conc. HCl	10	48	30°	S-6896	+++
Conc. HCl	20	48	30°	S-6896	+++
Conc. HCl	20	48	30°	S-6896	o - trace
Conc. H <sub>2</sub> SO <sub>4</sub>	10	48	30°	S-6896	o
Conc. H <sub>2</sub> SO <sub>4</sub>	10	24	30°	S-6896	o
Conc. H <sub>2</sub> SO <sub>4</sub>	10	16	30°	S-6896	Trace
Conc. H <sub>2</sub> SO <sub>4</sub>	10	8	30°	S-6896	+
Conc. HNO <sub>3</sub>	5	48	30°	S-6896	+ - +++
Conc. HNO <sub>3</sub>	10	48	30°	S-6896	+ - ++
Glacial acetic	20	48	30°	S-6896	++++
90% Formic	20	48	30°	S-6896	+ - ±

insolubility of hemosiderin in aniline, pyridine and 4 per cent quinine in chloroform and the slow solubility of malaria and formalin pigments in these reagents (3-14 days).

In a study of melanosis of the appendix,<sup>16</sup> I used extraction for 2 hours with 20 per cent sulphuric acid to exclude hemosiderin, and we have generally relied on similar extractions with 20 per cent sulphuric acid or a hydrochloric acid alcohol containing about 2.5 per cent hydrogen chloride to remove hemosiderin.

In the summer of 1937 a section of bone marrow which had been decalcified 2 or 3 days in a 20 per cent formic acid-sodium citrate fluid was included among several sections being stained for iron by the ferrocyanide method, and a considerable amount of hemosiderin was demonstrated.



This finding raised a question as to the degree and rapidity of solution of hemosiderin in acids. Accordingly, a series of experiments was initiated to explore the problem, primarily in connection with establishing reliable technical procedures for our own use.

In the first experiment a series of sections of a heavily hemo-

TABLE II

*Hemosiderin Remaining in Paraffin Sections of Formalin-Fixed Material from a Skin Tumor and a Liver from a Case of Hemochromatosis after Varying Exposures to Various Acids*

Stock acid	Dilution	Time	Temperature	Material	Amount of hemosiderin remaining
Conc. H <sub>2</sub> SO <sub>4</sub>	%	hrs.	C.		
	10	1	37°	S-6896	+
				A-1228	++
Conc. H <sub>2</sub> SO <sub>4</sub>	10	2	37°	S-6896	±
				A-1228	+
Conc. H <sub>2</sub> SO <sub>4</sub>	10	4	37°	S-6896	Trace
				A-1228	Trace
90% Formic	20	8	37°	S-6896	+++
				A-1228	++++
90% Formic	20	16	37°	S-6896	± most brown
				A-1228	+ most brown and in portal area
90% Formic	20	24	37°	A-1228	+ most brown and in portal area
Glacial acetic	20	48	37°	S-6896	++
				A-1228	++ - +++
Conc. HCl	10	8	37°	S-6896	+ part brown
				A-1228	++ part brown
Conc. HCl	..	16	37°	S-6896	± part brown
				A-1228	+ most brown and in portal area
Conc. HCl	..	24	37°	S-6896	± part brown
				A-1228	± most portal

siderotic cutaneous sarcoma, fixed routinely in 10 per cent formalin, was utilized. The paraffin sections were brought to water as usual and then soaked in acid solutions for varying periods. Sections were then washed in water and stained by Dr. Maude Abbott's modification of Perls' reaction (Mallory and Wright,<sup>8</sup> 1924, p. 207), using dilute fuchsin as a counterstain. The results are presented in Table I.

Table I shows that 20 per cent hydrochloric acid for 48 hours at about 30°C., or 10 per cent sulphuric acid for 16 hours, barely removed all the demonstrable iron, and that 10 per cent nitric

TABLE III  
*Persistence of Hemosiderin in Formalin-Fixed Tissues from Various Cases on Soaking in Sulphuric and Other Acid Solutions*

Time in acid at 25°C.	A-1747 Brown induration of lung (a)					10% Sulphuric acid					A-1695		
	5% oxalic	20% acetic	HCl alcohol	20% H <sub>2</sub> SO <sub>4</sub>	10% H <sub>2</sub> SO <sub>4</sub>	A-1743 Lung CPC(a)	A-1735 Lung CPC(a)	A-1716 Spleen	A-1593 Liver cirrhosis	A-1367 Spleen (b)	Liver Kc(b)	Spleen (c) pulp (b)	Lymph node (c)
Control	++	++	++	++	++	++	++	++	++	++	++	++	++
½ hr.	++	+	++	++	++	++	++	++	++	++	++	++	++
1 hr.	++	..	++	++	++	++	++	++	++	++	++	++	++
2 hrs.	++	..	++	++	++	++	++	++	++	++	++	++	++
4 hrs.	++	..	++	++	++	++	++	++	++	++	++	++	++
8 hrs.	++	..	++	++	++	++ (*)	++	++	++	++	++	++	++
16 hrs.	++	..	++	++	++	++	++	++	++	++	++	++	++
1 day	++	++	++	++	++	++	++	++	++	++	++	++	++
2 days	++	++	++	++	++	++	++	++	++	++	++	++	++
3 days	++	++	++	++	++	++	++	++	++	++	++	++	++
4 days	++	++	++	++	++	++	++	++	++	++	++	++	++
5 days	++	++	++	++	++	++	++	++	++	++	++	++	++
7 days	++	++	++	++	++	++	++	++	++	++	++	++	++

Kc = Kupffer cells.

CPC = Chronic passive congestion.

(a) = Some carbon present in addition to hemosiderin.

(b) = + formalin pigment in hemolyzed areas.

(c) = Some brown granular iron-free pigment.

(\*) = Unexplained irregularity.

acid and 20 per cent formic acid did not quite remove it all in 48 hours, and that 20 per cent acetic for 48 hours was almost without appreciable effect.

The next experiment was made at 37°C. and showed more rapid removal of demonstrable iron. However, often part of the brown pigment remained, though it failed to give the ferrocyanide reaction. The results are shown in Table II.

Table II shows that 4 hours at 37°C. in 10 per cent sulphuric acid removed practically all of the pigment, that 10 per cent hydrochloric acid or 20 per cent formic acid removed most of the iron from the pigment in 16 to 24 hours at 37°C. but left much brown pigment, and that acetic acid was a little more active in pigment removal or iron removal than at 30°C.

Further human formalin-fixed material from 7 cases was selected for testing to see whether there was any great variation in acid solubility of hemosiderin in various cases. One of these was used also to see whether longer exposure to 20 per cent acetic acid would be effective and to test the solvent action of oxalic acid on hemosiderin.

Table III shows that the limits of persistence of hemosiderin in 10 per cent sulphuric acid at room temperature (25°C.) were 16, 16, 4, 24, 8, 8 and 16 hours for the 7 cases, 4 hours in 20 per cent sulphuric, and 2 hours in 5 per cent oxalic acid in the 1st case. Hydrochloric acid alcohol (2.5 cc. HCl in 100 cc. 70 per cent alcohol) and 20 per cent acetic acid were very slow, still showing some hemosiderin after 15 and 30 days respectively.

Apparently iron-containing pigment in formalin-fixed material is relatively resistant to acids.

The next experiment was to test the solubility of the pigment in various acid-containing fixatives applied to fresh unfixed tissue from the spleens of guinea pigs previously given several intraperitoneal inoculations with sheep erythrocytes. The results are presented in Tables IV, V and VI.

From these tables it seems evident that formalin protects hemosiderin against the solvent action of formic and acetic acids, but not sulphuric, while Zenker's potassium bichromate and corrosive sublimate mixture, which by itself preserves hemosiderin, fails to prevent solution of the iron from the pigment by formic, acetic and nitric acids.

TABLE IV

*Amounts of Hemosiderin Shown in One Spleen Fixed by Various Fixing Fluids*

Fixing solution	Time	Amount of hemosiderin remaining	Red cells
	<i>hrs.</i>		
10% aqueous formalin, neutral	48	+++	Preserved
10% formalin, 5% acetic in water	48	+++	Laked
10% formalin, 5% formic in water	48	+++	Laked
10 pts. formalin, 90 pts. 95% alcohol	24	+++	Partly laked
10% formalin, 5% acetic in alcohol	24	+++	Laked
10% formalin, 5% formic in alcohol	24	+++	Laked
Helly-Maximow (HgCl <sub>2</sub> 6, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 2.5, H <sub>2</sub> O 100, formalin 10)	22	+++	Preserved
Zenker (HgCl <sub>2</sub> 6, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 2.5, H <sub>2</sub> O 100, glacial acetic 5)	22	+ brown	Laked
		+ green	
		± brown	
Zenker-formic (HgCl <sub>2</sub> 6, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 2.5, H <sub>2</sub> O 100, formic 5)	22	+ green	Mostly laked
		+ blue	
Zenker-nitric (HgCl <sub>2</sub> 6, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 2.5, H <sub>2</sub> O 100, HNO <sub>3</sub> 5)	22	+++ brown	Partly laked

TABLE V

*Further Tests of Fixing Solutions on Hemosiderin in One Spleen*

Fixing solution	Time	Amount of hemosiderin remaining	Red cells
	<i>hrs.</i>		
Sublimate (HgCl <sub>2</sub> sat. aq. 85, H <sub>2</sub> O 15)	24	—	—
Sublimate-nitric (sat. aq. HgCl <sub>2</sub> 85, H <sub>2</sub> O 10, HNO <sub>3</sub> 5)	24	—	Laked
Sublimate-acetic (sat. aq. HgCl <sub>2</sub> 85, H <sub>2</sub> O 10, acetic 5)	24	—	Laked
Sublimate-formol-acetic (sat. aq. HgCl <sub>2</sub> 85, formalin 10, acetic 5)	24	++	Preserved
Sublimate-formol (sat. aq. HgCl <sub>2</sub> 85, formalin 10, H <sub>2</sub> O 5)	24	++	Preserved
Sublimate-sulphuric (sat. aq. HgCl <sub>2</sub> 85, H <sub>2</sub> O 10, H <sub>2</sub> SO <sub>4</sub> 5)	24	—	Laked
Sublimate-formol-sulphuric (sat. aq. HgCl <sub>2</sub> 85, formalin 10, H <sub>2</sub> SO <sub>4</sub> 5)	24	—	Slight hemolysis
Zenker-without-acetic (HgCl <sub>2</sub> 6, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 2.5, H <sub>2</sub> O 100 + 15)	18	++	—
Zenker-formol-hydrochloric (HgCl <sub>2</sub> 6, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 2.5, H <sub>2</sub> O 100, formalin 10, HCl 5)	18	+++	Subtotal hemolysis
Zenker-formol-acetic (HgCl <sub>2</sub> 6, K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 2.5, H <sub>2</sub> O 100, formalin 10, acetic 5)	18	++	Partial hemolysis

Following these fixation experiments paraffin sections from blocks of tissue fixed in fixatives not containing formaldehyde and showing preservation of hemosiderin were treated with acids for varying periods as in the first experiments and the results are presented in Table VII.

From this table it is evident that hemosiderin fixed with potassium bichromate and corrosive sublimate (Zenker-without-

TABLE VI

*Tests of Fixing Solutions on Preservation of Hemosiderin in One Spleen*

Fixing solution	Time	Amount of hemosiderin remaining	Red cells
	<i>hrs.</i>		
Picro-sublimate (5% $\text{HgCl}_2$ in sat. aq. picric acid)	24	$\pm$ blue + green + brown	Preserved
Picro-sublimate-sulphuric (5% $\text{HgCl}_2$ in sat. aq. picric + 5% $\text{H}_2\text{SO}_4$ )	24	+	Partial hemolysis
Picro-sublimate-formol (5% $\text{HgCl}_2$ in sat. aq. picric + 10% formalin)	24	+++	Preserved
Picro-sublimate-formol-sulphuric (5% $\text{HgCl}_2$ in sat. aq. picric, 10% formalin, 5% $\text{H}_2\text{SO}_4$ )	24	+	Partial hemolysis
Bouin (sat. aq. picric 75, formalin 20, acetic 5)	24	+ brown	Partial hemolysis
Carnoy (abs. alcohol 60, $\text{CHCl}_3$ 30, glacial acetic 10)	24	++	Laked
Formol-Carnoy (abs. alcohol 60, $\text{CHCl}_3$ 30, formalin 10)	2	+ green + brown	Partial hemolysis
Carnoy-formol (abs. alcohol 60, $\text{CHCl}_3$ 25, formalin 10, glacial acetic 5)	2	—	Partial hemolysis
Absolute alcohol	18	+ green	Laked
10% formalin	48	+++	Preserved
Formol-sulphuric (10% formalin, 5% $\text{H}_2\text{SO}_4$ )	48	++ brown	Laked

acetic) is materially less resistant to acid extraction than is formalin-fixed pigment, and that Carnoy-fixed pigment is intermediate in its resistance. The same differences in extracting power among sulphuric, formic and acetic acids as noted previously were seen in this series. Mercuric chloride, as previously indicated in the fixation experiments, apparently shows feeble extracting power, again more in the Zenker-without-acetic material than in the Carnoy-fixed specimens.

To confirm and extend these results a further series of rats

was injected subcutaneously with phenylhydrazine hydrochloride in single doses of 80 mg. per kg. and killed 4, 8, 16 and 24 hours, and 2, 3, 4 and 6 days later, and blocks of the spleens, kidneys and livers were fixed each by 7 fixation methods as follows: 85 per cent alcohol, 24 hrs.; Carnoy's fluid (absolute alcohol 6, chloroform 3, glacial acetic acid 1) 2 hrs.; corrosive sublimate (saturated  $\text{HgCl}_2$  85,  $\text{H}_2\text{O}$  15) 24 hrs.; Zenker-without-acetic, 24 hrs.; 10

TABLE VII

*Test of the Solubility of Hemosiderin in Paraffin Sections in Various Reagents at 25°C. after Fixation with Fluids not Containing Formalin*

Fixation	Extracting agent	Time	Amount of pigment remaining	Color with acid ferrocyanide	Erythrocytes color with ferrocyanide
		<i>hrs.</i>			
Zenker- without- acetic	10% H <sub>2</sub> SO <sub>4</sub>	4	—	—	Greenish
		8	—	—	—
		16	—	—	Faintly greenish
		24	—	—	—
	20% formic	24	—	—	—
		48	—	—	Pale blue
	20% acetic	24	++	Blue	—
		48	++	Blue	—
	Saturated HgCl <sub>2</sub>	24	+	Blue	—
		48	+	Faint blue	—
Carnoy's	10% H <sub>2</sub> SO <sub>4</sub>	4	±	Blue	Laked
		8	—	—	Laked
		16	—	—	Laked
		24	—	—	Laked
	20% formic	24	+	Blue-green	Laked
		48	+	Blue, green, brown	Laked
	20% acetic	24	++	Blue	Laked
		48	++	Blue	Laked
	Saturated HgCl <sub>2</sub>	24	++	Blue-green	Laked
		48	++	Blue-green	Laked

per cent formalin, 48 hrs.; 10 per cent formalin and 5 per cent formic acid, 48 hrs.; and the last at 56°C. for 48 hours.

Hemosiderin was best preserved by potassium bichromate and corrosive sublimate (Zenker-without-acetic), 10 per cent formalin, and corrosive sublimate, fairly well with Carnoy's fixative, somewhat less with 85 per cent alcohol, particularly in the central portions of the blocks, and about one-third with the formic acid-formalin at room temperature. The last reagent acting at 56°C. for 48 hours not only removed all of the hemosiderin, but seriously impaired nuclear staining as well.

In passing it may be noted that appreciable quantities of iron-

reacting pigment appeared in the spleen in 16 hours and in the liver in 48. Only 1 animal, killed after 4 days, showed blue granules with ferrocyanide in the epithelium of the renal convoluted tubules.

Sections fixed in several of the foregoing solutions were further tested for the solubility of the hemosiderin in 10 per cent sulphuric acid at 25°C. and at 37°C. (Tables VIII and IX).

At 25°C. small amounts of pigment coloring blue to green with ferrocyanide were evident after 1 hour in the Carnoy and

TABLE VIII

*Extraction of Pigment by 10% Sulphuric Acid at 25°C. after Various Fixatives*

Fixative	No. of rat	Organ	10% H <sub>2</sub> SO <sub>4</sub> at 25°C.			
			1 hr.	2 hrs.	4 hrs.	Control
Carnoy's	38 2d	Liver	—	—	—	±
		Spleen	± blue s.c.	—	—	+
	43 3d	Liver	—	—	—	+++
		Spleen	± blue-green	—	—	+
HgCl <sub>2</sub>	38 2d	Liver	—	—	—	±
		Spleen	—	—	—	+++
	43 3d	Liver	—	—	—	+++
		Spleen	—	—	—	+++
Zenker-without-acetic	38 2d	Liver	± brown	± brown	—	+
		Spleen	± brown-green	—	—	+++
	43 3d	Liver	± brown	—	—	+++
		Spleen	± gray-brown	—	—	+++
10% formalin	38 2d	Liver	—	—	—	±
		Spleen	—	—	—	+++
	43 3d	Liver	—	—	—	+++
		Spleen	—	—	—	+++

Zenker-without-acetic material, but not later, while at 37°C. some brown iron-free pigment remained for 1 and 2 hours but no free iron remained.

Comparing Table VIII with Table II, it is seen that the pigment in the last experiment, fixed within a few days of its appearance, was more readily removed by sulphuric acid than was the probably older pigment in the human material.

Following this indication that recently formed hemosiderin might be more readily soluble in acids than older pigment, further experiments with phenylhydrazine \* were carried out.

\* Mice averaging 21 gm. were used. A dose of 5 mg. (240 mg. per kg.) of phenylhydrazine hydrochloride killed 92 per cent of 25 mice in less than 24 hours, most of them in 30 minutes. Doses of 2 and 3 mg. (95 and 145 mg. per kg.) were

With phenylhydrazine, traces of iron-containing pigment appear in the spleen in 24 hours. Very considerable amounts are evident in the splenic follicles in 48 hours, less in the pulp. The intrafollicular pigment persists only for 5-6 days, and reacts with ferrocyanide throughout that period. Considerable amounts of brown pigment which fail to react with ferrocyanide persist in the pulp and less in the follicles for as long as 25 days. This pigment also fails to react with potassium ferricyanide.

On testing sections of those spleens showing considerable amounts of iron-reacting pigment for solubility of the pigment

TABLE IX

*Extraction of Pigment by 10% Sulphuric Acid at 37°C. after Various Fixatives*

Fixative	No. of rat	Organ	10% H <sub>2</sub> SO <sub>4</sub> at 37°C.				
			1 hr.	2 hrs.	3 hrs.	5 hrs.	Control
Carnoy's	272 4d	Liver	—		—	—	++
		Spleen		—	—	—	+
	271 6d	Liver	—	—	—		++
		Spleen	—	—	—	—	++
HgCl <sub>2</sub>	272 4d	Liver	—	—			++
		Spleen		—	—	—	++
	271 6d	Liver			—	—	±
		Spleen	—		—	—	++
Zenker- without- acetic	272 4d	Liver	± brown	± brown			±±
		Spleen		—	—	—	++
	271 6d	Liver		± brown	—	—	++
		Spleen	++ brown		—	—	++
10% for- malin	272 4d	Liver	++ brown	++ brown			++
		Spleen		—	—	—	++
	271 6d	Liver		—	—	—	++
		Spleen	++ brown		+ brown	—	++

in 10 per cent sulphuric acid at room temperature, it was found that in all animals the intrafollicular iron-containing pigment was removed in one-half to 1 hour, or perhaps persisted for some hours as an iron-free brown pigment. In any case, no iron reaction was positive after more than 1 hour in acid.

As iron-containing pigment disappeared in this series in a short period no indication was given as to alteration of acid solubility of hemosiderin with age.

better tolerated, 92 and 78 per cent respectively surviving for 24 hours, and 84 and 62 per cent for 48.



A series of 7 formalin-fixed spleens containing considerable quantities of hemosiderin in the pulp, from animals intoxicated with organic selenium compounds for periods varying from 25 to 380 days were studied in respect to the acid solubility of the pigment. The exposures to organic selenium were 25, 32, 156, 342, 372, 373 and 380 days, the time necessary for solution of the iron-containing pigment in 10 per cent sulphuric acid at room temperature was 2, 2, 2, 8, 2, 8 and 4 hours.

A series of 24 formalin-fixed spleens from rats intoxicated with sodium vanadate for periods varying from 2 to 53 days were tested as to the solubility of the pigment in 10 per cent sulphuric acid at room temperature. Regardless of the length of exposure to vanadium, the pigment disappeared in approximately 4 hours in all animals.

Apparently the age of the pigment is not a determining factor in its resistance to solution in 10 per cent sulphuric acid.

From the foregoing, it was noted that the iron of hemosiderin is quickly soluble in oxalic acid and very slowly if at all soluble in acetic acid. Standard references on solubilities of inorganic salts note that ferric oxalate is highly soluble in water and basic ferric acetate insoluble, while ferrous oxalate is insoluble in water and ferrous acetate quite soluble. This would appear to indicate that the iron content of hemosiderin is largely ferric.

In studies on the toxicology of sodium formaldehyde sulfoxylate done in 1933, I found that none of the copious iron-containing pigment reacted directly with potassium ferricyanide. Gömöri<sup>17</sup> also noted that he has never obtained a direct reaction with ferricyanide and concludes that hemosiderin does not contain ferrous compounds thus demonstrable.

Having noted that ferrous sulphate in solution is almost immediately converted to the ferric form by hydrogen peroxide, I decided to try various oxidizing and reducing agents on heavily hemosiderotic lung, liver and spleen, considering that the first would theoretically contain the least ferrous iron, and the two latter more, if any such were to be found. The results are presented in Table X.

From this table it is noted that there is no evident increase in demonstrable ferric iron on oxidation with  $\text{H}_2\text{O}_2$ , that no ferrous iron is demonstrable with ferricyanide, even after reduction with

TABLE X  
*The Effect of Oxidizing and Reducing Agents on the Reactions of Hemosiderin after Formalin Fixation  
 with Potassium Ferrocyanide and with Potassium Ferricyanide*

Preliminary treatment	Results with ferrocyanide			Results with ferricyanide		
	Amount	Form	Color	Amount	Form	Color
None	+++	Granular	Dark blue	++	Granular	Brown
Hydrogen peroxide USP 1 hr. 25°C.	+++	Granular	Dark blue	++	Granular	Brown
4% elon* 1 hr. 25°C.	+++	Granular	Dark blue	++	Granular	Brown
4% hydroquinone 1 hr. 25°C.	+++	Granular	Dark blue	++	Granular	Brown
10% pyrogallol in half saturated sodium carbonate 1 hr. 25°C.	++	Granular	Dark blue	++	Granular	Brown
10% sodium sulphite 1 hr. 25°C.	++	Granular	Dark blue	++	Granular	Brown
Yellow ammonium sulphide sol. in 3 parts alcohol 17 hrs. 25°C.	++	Granular	+ blue halos	++	Granular	+ blue halos

\* Elon = trade name for monomethylparamidophenol sulphate, a photographic developer.

elon, hydroquinone, alkaline pyrogallol or sodium sulphite, and that after treatment with ammonium sulphide part of the iron still reacts with ferrocyanide, and part with ferricyanide. The quantity in both cases is evidently less than with the direct ferrocyanide procedure or with the Quincke iron sulphide reaction, and marked diffusion of the blue stain out from the granules is noted.

This agrees completely with Gömöri's<sup>17</sup> findings and further indicates that, as part of the iron still reacts as ferric after the ammonium sulphide treatment, the Tirmann-Schmelzer sulphide Turnbull blue reaction affords an incomplete and partial demonstration of the iron content of the pigment.

### DISCUSSION .

Hemosiderin after fixation with formalin is rather slowly soluble in dilute acids, best in oxalic, then sulphuric, then nitric, formic and aqueous hydrochloric acids. It is very slowly soluble in hydrochloric acid alcohol and almost insoluble in 20 per cent acetic acid.

Higher concentrations of acid increase the speed of solution, and elevation of temperature facilitates solution of at least the iron component of hemosiderin.

Fixatives that contain acids but no formalin often completely remove hemosiderin or alter it so that brown pigment containing no demonstrable iron is produced. The presence of formalin in the fixative often protects hemosiderin against acetic, formic and hydrochloric acids, but not sulphuric. Alcohol is inferior to formalin as a fixative for hemosiderin.

Formalin in the fixing fluid tends to make hemosiderin more resistant to subsequent extraction with acids.

The alteration of the solubility of the iron in hemosiderin by certain fixatives as compared with others and with unfixed material indicates that iron exists in a protein combination. This concurs with the views of Hueck,<sup>5</sup> Schmidt,<sup>18</sup> Neumann,<sup>19</sup> and Arnold.<sup>20</sup>

That brown granular pigment containing no demonstrable iron may persist in areas of old hemorrhage after the disappearance of hemosiderin has often been noted. The present findings show that a similar pigment may be produced from hemosiderin by

the presence of acids in the fixing fluids, or by the action of warm acids on previously fixed material. This indicates that hemofuscin may be derived from hemosiderin by the loss of its iron content.

It seems clearly indicated that the iron of hemosiderin is in the ferric state. Even powerful reducing agents fail to reduce it to the ferrous form, and even after conversion into iron sulphide, part of the iron appears to remain in, or very promptly to revert to, the ferric state.

Considerable variation in the speed of solution of hemosiderin in acids is noted from case to case, and in experimental hemosiderosis with the mode of its production. The age of the pigment appears to have little influence on its speed of solution. Granule size has a material influence on speed of solution, coarser granules resisting longer.

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# THE POLARIZED LIGHT METHOD FOR THE STUDY OF MYELIN DEGENERATION AS COMPARED WITH THE MARCHI AND SUDAN III METHODS \*

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## INTRODUCTION

For many years the methods most commonly used for the demonstration of myelin degeneration in the peripheral and central nervous system have been the Weigert, the Marchi, the sudan III, and the scarlet red.

The use of the Marchi method has been criticized by numerous authors because of its capriciousness and because of the large amount of artefact frequently present which interferes considerably with correct diagnosis in cases of suspected early degenerative change. The Weigert, the sudan III and scarlet red methods, although apparently giving consistent results, have the disadvantage of not demonstrating the early phases of myelin change.

In connection with the study of vitamin B<sub>1</sub> deficiency in the rat, we have had occasion to examine the nervous tissues of approximately 500 animals. In these studies the variability of the Marchi method, and the unsuitability of the sudan III and scarlet red methods for the detection of early myelin change in the nerves of rats were thoroughly confirmed.

The results obtained by Sutton, Setterfield and co-workers<sup>1-4</sup> with polarized light in the study of early myelin changes in degenerating nerves indicated that it might be advantageously used in the study of nerves from vitamin B<sub>1</sub> deficient animals. As the method had not been generally used it seemed desirable to subject it to a critical study in which both normal and transected nerves would be examined by the polarized light method as well as by the conventional Marchi and sudan III methods. The results confirm and extend the findings of Sutton and his co-workers, and it is believed that the further observations made are fundamental and

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will be of value to anyone wishing to use the method in similar studies.

### EXPERIMENTAL

The nerves for this study were obtained from normal rats 6 to 8 weeks of age and approximately 150 gm. in weight. They had been fed the complete ration used in the stock colony.<sup>5</sup> The right and left lumbosacral and sciatic nerve trunks from 142 rats were studied. Of these, 71 rats were used for studies of the normal nerve, 50 of which were studied by the polarized light method, 11 by the Marchi method, and 10 by the sudan III method. In the remaining 71 rats the right nerves were transected while the left nerves were left intact and studied as normal controls; in this group 28 were studied by the polarized light method, 23 by the Marchi, and 20 by the sudan III method.

For the study of progressive degenerative changes due to transection of the nerve, the animals were lightly anesthetized with ether, the sciatic trunk was cut as high up in the leg as possible, and about 1 mm. of the nerve was removed. When the desired length of time after transection had elapsed, these animals were killed by decapitation. The progress of degenerative change was studied at intervals of 24, 48, 72, 120 and 216 hours after transection. There was no evidence of infection during the interval between transection and killing. The animals used for studies of the normal nerve were either decapitated or killed with chloroform.

After the skin was removed from over the posterior extremities, the muscles were cut away to bare the lumbosacral and sciatic trunks of the right and left extremities so that rapid fixation would take place. As it was considered undesirable to remove the nerves from the legs before fixation, the entire pelvis and extremities were placed in the desired fixative.

The tissues used for the polarized light and sudan III methods were fixed in 10 per cent neutral formalin (Merck) for 48 hours, the fixative being changed at the end of 24 hours. The nerves were removed from the extremities at the end of 48 hours and stored in fresh 10 per cent neutral formalin until sectioned.

The tissues used for the Marchi method were fixed in Müller's fluid for 7 days, the fixative being changed daily for the first 3 days. The nerves were removed from the legs at the end of the 2nd day. After fixation the nerves were placed in Marchi's fluid for 7 days,



washed, dehydrated, and embedded in pyroxylin as rapidly as possible.\*

Before the nerves were sectioned they were arbitrarily divided into three pieces: the "proximal" portion consisted of the lumbosacral trunk from its exit from the vertebral column to the head of the femur; the "medial" portion consisted of the sciatic trunk from the head of the femur to the knee joint; and the "distal" portion extended from this point to the heel. In transected nerves only the portions distal to the cut were studied. These corresponded to the "medial" and "distal" portions of the normal nerve.

For study by the polarized light method, frozen sections were made on a clinical microtome at 10, 20 and 35 microns. The sections were cut into distilled water and immediately mounted in glycerin or glycerin jelly.<sup>6</sup> Because of the clearing action of the mounting medium, visual observations were made about 30 minutes after mounting in order to secure as constant a picture as possible. With only a few differences, which will be discussed later in this paper, the technic of visual observation was essentially the same as that described by Sutton, Setterfield and Krauss.<sup>1</sup> As the mounts were not permanent, microphotographs were taken within 60 minutes after mounting and constituted the only permanent record of the section. The microphotographs were taken at 500 diameters at the point of greatest birefringence between crossed Nicols.

For study by the sudan III method, frozen sections were made at 20 microns,\* stained, and mounted in glycerin jelly. The nerves prepared by the Marchi method were cut at 20 microns,† dehydrated, cleared in xylol and mounted in dammar.

## RESULTS

### *The Polarized Light Method*

*General Observations:* During the early part of this study it was noted that when normal nerves were cut at 20 microns, numerous

\* Comparison of frozen sections of unembedded nerves prepared by the Marchi method with those embedded in pyroxylin indicated that this rapid dehydration and embedding did not alter the resulting picture.

† Thinner sections (10 microns) were studied, but in this case the thickness of sections showed no apparent differences or advantages, and sections cut at 20 microns were used throughout this study.

fibers at the periphery and occasionally at the ends showed marked differences in structure from those in the body of the section. There was considerable darkening of the fibers as compared with the body of the section, and the proportion of the myelin sheath to the axis cylinder was greater. Nodes of Ranvier and incisures of Schmidt-Lantermann were also more frequent and more clearly seen. It was also observed that the same structure was exhibited by isolated fibers, the more distal portions of the nerve trunk, and occasional sections which were thinner than usual. It seemed apparent that the thickness of the section had some bearing on these differences, and a study of the effect of thickness on the details of structure was made.

In order to determine the effect of the thickness of the sections on the visibility of structural details, a series of sections 10, 20 and 35 microns in thickness was prepared. Figures 3 to 5 illustrate the results of this study in normal nerves, and Figures 6 to 8 show the effect of thickness in transected nerves. The structure of the fibers in sections cut at 10 microns (Fig. 1) more closely resembled the structure of isolated fibers (Fig. 1) than those in sections cut at 20 or 35 microns (Figs. 2 and 3). The sections cut at 10 microns constituted roughly a single layer of fibers, and the amount of birefringent material was reduced to a minimum. Visibility was thus increased and fibrillar and interfibrillar structures were more clearly depicted. The increase in birefringent material (myelin sheaths) in the thicker sections caused a haze, which almost completely obliterated any details of structure in the sections cut at 35 microns. It is interesting to note in this connection that in taking microphotographs of sections of different thickness, about one-fifth the exposure necessary for the sections cut at 10 microns was adequate for those cut at 35. Variation in the size of individual fibers within a given section could be clearly seen in the thinner sections (10 microns), whereas in the thicker ones (20 and 35 microns) the haziness often obliterated them altogether.

After considerable study of sections of different thickness, the sections cut at 10 microns were selected as offering the truer representation of the individual fibers and their relations to each other. This thickness was used throughout the remainder of the study.

Since it was desired to apply this method to the study of the peripheral nerves of vitamin B<sub>1</sub> deficient animals where autopsy

material was desired, a study was made to determine the effect of decapitation as compared with killing with chloroform. No apparent differences due to the method of killing were noted in any of the nerves studied.

In variation from the method of Sutton, Setterfield and Krauss,<sup>1</sup> some of the sections were mounted in glycerin jelly<sup>6</sup> rather than in glycerin. This semisolid medium allowed the sections to be safely photographed with the microscope in the horizontal position; it also had the advantage of making it possible to weight the coverglass, and as the material hardened the section remained flattened. A further advantage was that the nerve did not clear so rapidly as when mounted in glycerin. A comparison of sections mounted in both mediums showed that although the glycerin jelly gave a somewhat darker preparation, this did not interfere in any way with the study of the nerve.

*The Normal Nerve and its Variations:* In 10 micron sections the individual fibers of the nerve did not always follow the parallel course seen in preparations cut at 20 microns. However, the slight waviness seen at times would hardly be mistaken for the tortuosity present in degenerating fibers. Individual fibers were quite regular in size along their course except at the nodes of Ranvier, but differed in size as compared with other fibers in the same area. The fibers seen in the "medial" and "distal" portions of the nerve were usually smaller in diameter, and the proportion of the myelin sheath to the axis cylinder was usually less, although more variable, than in the "proximal" portion.

The myelin sheath was usually birefringent, brilliantly clear at the points of greatest birefringence, and rarely contained isotropic materials. All the materials in the sheath, however, did not become dark at the points of extinction; this gave the section a finely granular appearance. The nodes of Ranvier varied considerably in size and in structure, and were at times very granular. Part of the granulations remained light at the points of extinction. In the greater number of cases, however, the nodes showed club-shaped enlargements which were similar to those previously found in Busch preparations.<sup>5</sup> The incisures of Schmidt-Lantermann were not always present but were quite frequently seen. When visible, they were irregularly placed, their apices varied in direction, and they contained variable amounts of isotropic material. Although

they had been observed at all thicknesses, they were more frequently seen in the 10 micron (Fig. 1) than in the thicker sections (Figs. 2 and 3). The axis cylinders were isotropic and varied somewhat in size. Their borders were usually sharply defined, but at times were mildly roughened. Tortuosity of the axis cylinder was not observed in normal fibers. Interfibrillar connective tissue was isotropic, as were perineural fat deposits. The interrelationship of fibers could often be seen better by uncrossing the Nicol prisms and cutting down the light.

*The Transected Nerve:* The earliest period at which degenerative change was studied in these experiments was 24 hours after transection, at which time marked and consistent changes were observed. Although the severity of the degenerative change observed at any given interval after transection varied somewhat in different nerves, the progress of the degeneration after 24 hours was essentially as shown by Sutton, *et al.*,<sup>1</sup> and will not be repeated here. However, in the transected nerve as well as in the normal, thickness of the section was an important consideration. Although the edema consistently present in the transected nerves tended to separate the fibers to a variable extent and thus show more isolated fibers than in normal nerves, the thicker sections were hazy and details of structure were at times markedly obscured (Figs. 4-6).

In the earlier stages of the degenerative change localized areas of swelling and apparent fragmentation of the fibers were noted which did not become blackened at the points of extinction. Either this material was not birefringent, or else it was composed of numerous droplets of still normal myelin which did not allow rotation of the section to cause extinction because of its multiple refractive surfaces.

It was often of very great advantage when confronted with hazy undifferentiated threads of fibrillar tissue which seemed partially isotropic, or with a black space between the nerve fibers, to uncross the analyzer prism and cut down the light by lowering the substage condenser. By this means one could distinguish between a tangentially cut fiber which was discontinuous and showed its thready nature, and an isotropic portion of a fiber which usually showed the sheath elements and its continuity with the birefringent portions of the fiber. It could be determined in the same manner

whether a black structureless space between fibers was a split in the section or a darkened area composed of completely isotropic fibers (Figs. 16-19).

The above procedure was also of value in ascertaining whether the axis cylinder of a nerve fiber was swollen or whether there was periaxillar accumulation of isotropic material. In some cases fusiform areas of blackening were seen along the course of the nerve fibers. By uncrossing the Nicols one could often determine whether this represented an accumulation of degenerated myelin or a Schwann cell.

During the progress of the degenerative change many fibers were seen which assumed a "sausage-link" structure, apparently indicating fragmentation. However, when such fibers were viewed with the analyzer uncrossed numbers of them were found which were still contained within an intact sheath (Figs. 16 and 17).

The nerves of 8 animals (16 sciatics) were studied in the fresh state without previous fixation. Frozen sections were made 10 or 20 microns in thickness and mounted in glycerin. The sections were cut into distilled water but remained in it only a few seconds before being placed on a slide and mounted. Study of these nerves revealed a completely different picture from that observed in the fixed nerves (Figs. 13-15). The fibrillar structure was not clearly seen and the tissue reacted only minimally or not at all to rotation of the stage. Degenerated nerves showed more similarity to the fixed nerves than did the normal nerves. A comparison of sections cut at 10 and 20 microns showed that, as in the case of fixed nerves, the increase in myelin caused considerable distortion (Figs. 13 and 14).

### *The Marchi and the Sudan III Methods*

Normal nerves prepared by the Marchi technic exhibited a marked variation in the type and amount of blackened material present. This variability was so great that correct diagnosis was at best exceedingly difficult and uncertain. Nerves transected for 96 hours were seen which showed very little staining with osmic acid, while normal nerves frequently contained copious amounts of blackened droplets. In the transected nerve the first stage at which degenerative change could be definitely ascertained was 72 hours after transection (Fig. 9). The amount of blackened

material indicative of degeneration present at any given interval varied more than was indicated in comparable preparations studied by the polarized light method. Segmentation and fragmentation could be seen clearly as the light was decreased by lowering the substage condenser in areas that showed no blackening with osmic acid. Sections prepared by the Marchi and polarized light methods at intervals of 72 and 120 hours after transection are compared in Figures 9-12. In contrast to the polarized light method only myelin changes are seen in the Marchi preparations.

The normal nerves prepared by the sudan III method showed no evidence of staining except for perineural fat deposits which were intensely dyed. The earliest definite staining of degenerated fibers in transected nerves was 120 hours after transection, although segmentation and other fibrillar changes could be seen clearly long before this time despite the lack of staining.

### DISCUSSION

The results obtained in studies of normal and degenerated nerves by the polarized light method show that it is a valuable supplement to the older methods for the demonstration of myelin change. Its chief disadvantages are the comparatively small amount of critical study to which it has been subjected and the necessity of taking routine microphotographs in order to obtain permanent records. As with all new technics, this method demands that careful and critical observations be made until one is thoroughly familiar with its intricacies. On the other hand, it has many advantages over the older methods. It is simple, rapid and consistent. Technical manipulations are reduced to a minimum, thus lessening the possibility of producing artefacts. Both axis cylinder and myelin sheath changes are shown in the same preparation. Moreover, myelin sheath changes after transection of nerves can be demonstrated much earlier by the polarized light method than by either the Marchi or sudan III methods.

The thickness of the section is very important in the polarized light method. It is believed that sections cut at 10 microns offer a truer representation of the structure of the individual fibers and their interrelations than do those cut at 20 or 35 microns. Since those cut at 10 microns represent roughly a single layer of fibers, the haziness due to the birefringent material is minimal and the

details are more clearly seen than in the thicker sections. The use of a microtome which is more accurate than the clinical microtome would be advantageous.

Although only a minimal amount of evidence is at hand, it indicates that the structures observed in normal and degenerated nerves by the polarized light method are to some extent the result of fixation in formalin. The myelin sheaths of fresh (unfixed) nerves exhibited only slightly the property of birefringence, although in two animals, whose sciatic nerves had been transected for 216 hours, the typical "Maltese cross" effect (Fig. 15) consistently seen in degenerated nerves fixed in formalin was visible but reacted only slightly to rotation of the stage. This effect of formalin, however, does not detract from the usefulness of the method.

#### SUMMARY

1. A study has been made of the merits of the polarized light method as compared with the Marchi and sudan III methods in demonstrating myelin degeneration due to transection of the peripheral nerves of the rat.

2. The polarized light method was found to be rapid and accurate. The changes depicted were consistent and did not depend on numerous technical manipulations for their demonstration. Both myelin sheath and axis cylinder changes were visible in the same preparation.

3. As compared with the polarized light method, the Marchi method gave very inconsistent results. The sudan III method was consistent but failed to reveal the early changes following transection.

4. Marked and advanced changes were shown by the polarized light method in nerves which had been transected only 24 hours. The earliest degeneration shown by the Marchi method was 72 hours after transection and by the sudan III method 120 hours after transection.

5. The thickness of the section influenced the structural detail observed by the polarized light method. Sections 10 microns in thickness showed more detail than thicker sections.

6. It was found to be advantageous to uncross the analyzer prism for determining the continuity of fibers which appear seg-

mented, for distinguishing between edema of the axis cylinders and periaxillar accumulation of isotropic material, and for revealing the presence of isotropic fibers masked by the crossed Nicols.

7. Sections of fresh unfixed normal or degenerating nerves, when viewed by polarized light, presented an appearance considerably different from that of fixed nerves. This does not detract, however, from the usefulness or reliability of the method.

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## DESCRIPTION OF PLATES

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### PLATE 42

All microphotographs were taken at a magnification of 500 diameters. Except for Figs. 9, 11, 17 and 19, all microphotographs were taken in polarized light at the point of greatest birefringence between crossed Nicols.

Wratten metallographic plates were used with a contrast developer (Eastman D-19) for photographs of preparations studied by the polarized light method. Wratten "M" plates were used for the photographs of the Marchi preparations.

FIG. 1. Normal nerve. Microphotograph of the "proximal" portion showing structural details as seen in a section cut at 10 microns. Note the fibrillar detail, the proportion of myelin sheath to axis cylinder, the presence of numerous incisures of Schmidt-Lantermann, and the nodes of Ranvier. Compare with Figs. 2 and 3. Polarized light method.

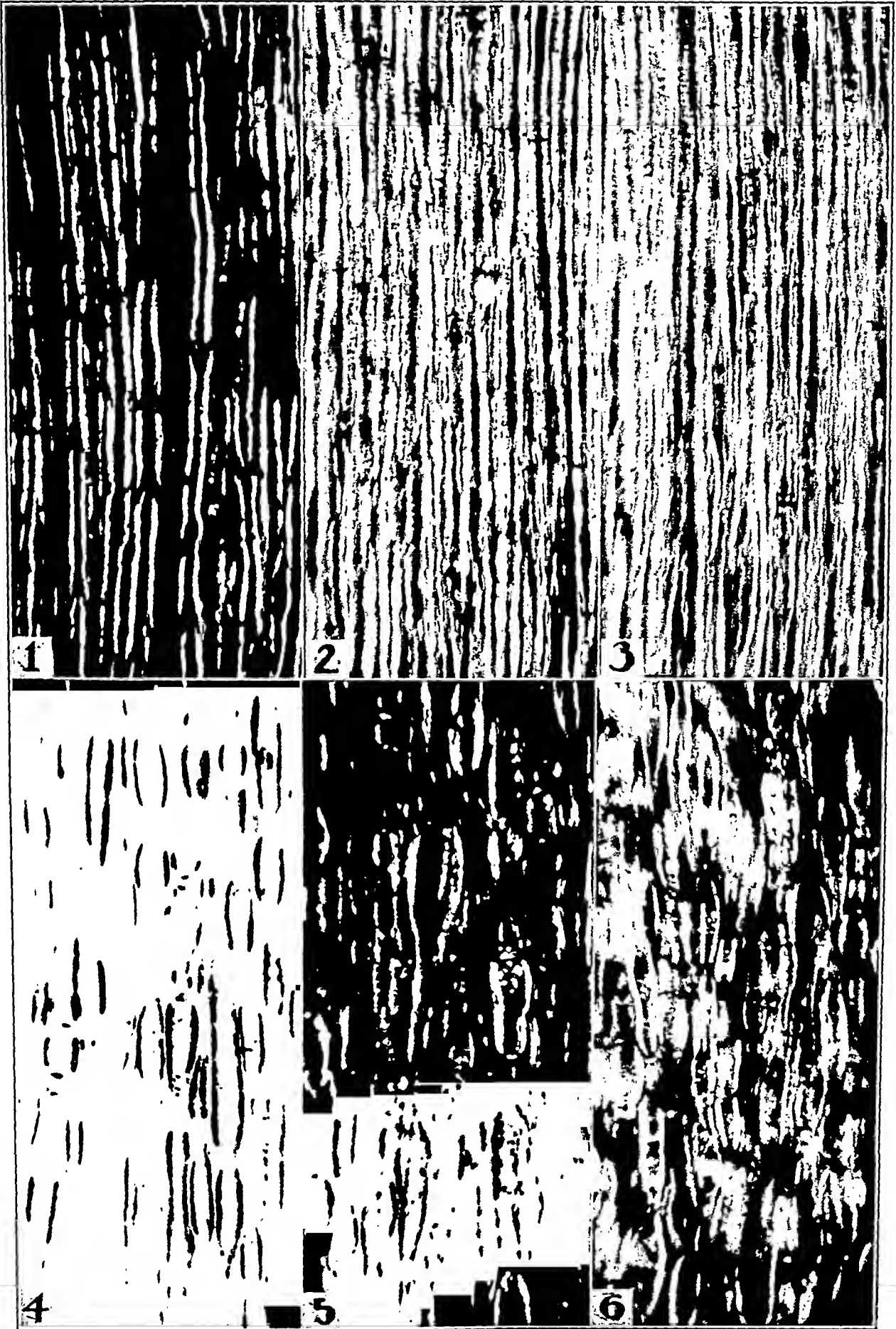
FIG. 2. Normal nerve. Microphotograph of the same portion of the same nerve shown in Fig. 1. Note the increase in haziness and loss of detail in sections cut at 20 microns. Polarized light method.

FIG. 3. Normal nerve. Microphotograph of the same portion of the same nerve as shown in Figs. 1 and 2. Note the almost complete loss of detail in sections cut at 35 microns. Compare the structure of the node of Ranvier in the middle right hand portion of the photograph with the nodes in Fig. 1. Polarized light method.

FIG. 4. Degenerated nerve. Microphotograph of a section of a nerve which had been transected for 48 hours. Edema, segmentation and increase in isotropic material in the individual fibers are clearly seen. Section cut at 10 microns. Compare with Fig. 1. Polarized light method.

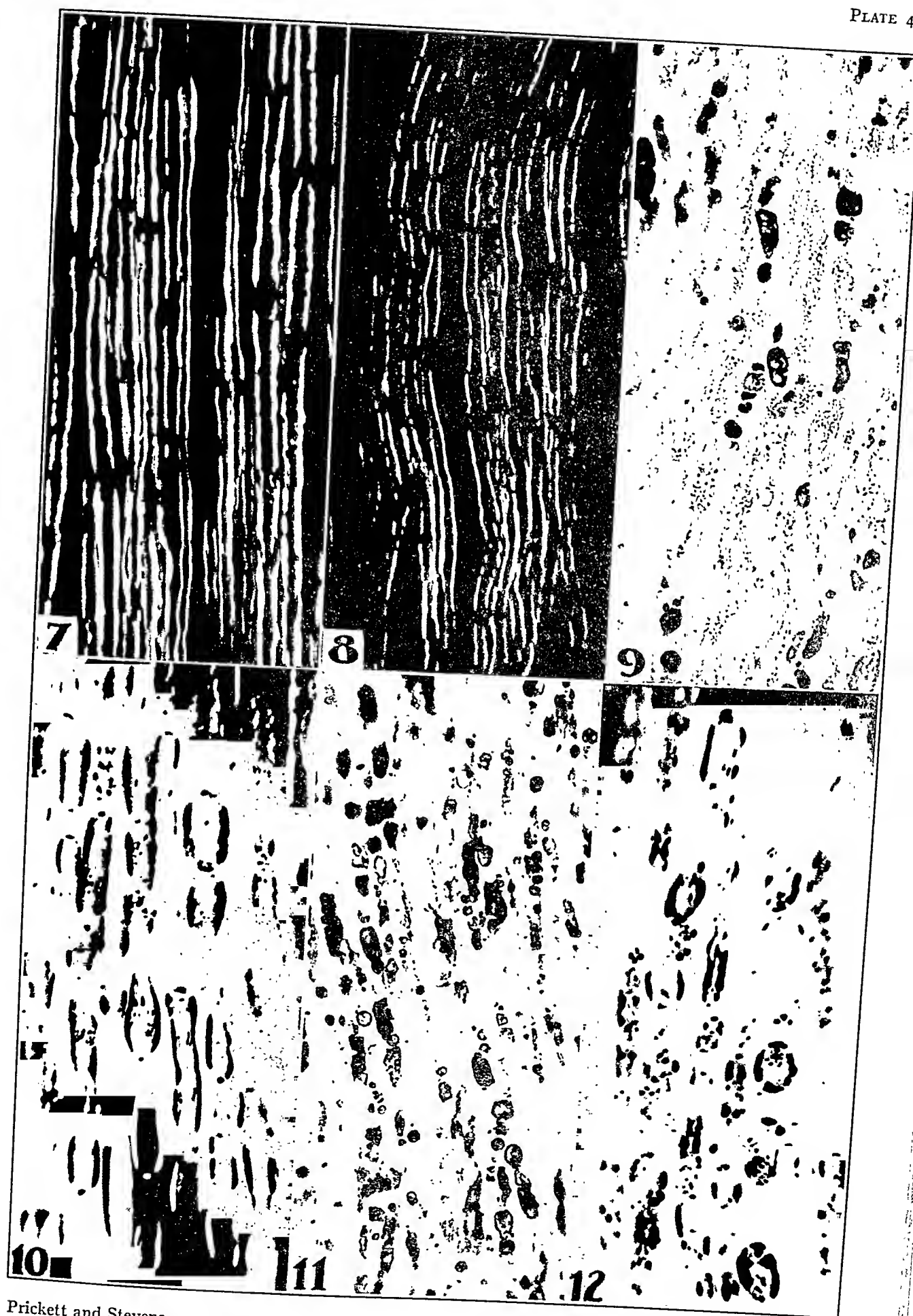
FIG. 5. Degenerated nerve. Microphotograph of the same portion of the same nerve as shown in Fig. 4. Note the loss of detail and interference due to superimposed layers of fibers in sections cut at 20 microns. Polarized light method.

FIG. 6. Degenerated nerve. Microphotograph of the same portion of the same nerve shown in Figs. 4 and 5. Note increased interference and haziness in section cut at 35 microns. Polarized light method.



# PLATE 43

- FIG. 7. Normal nerve. Microphotograph of the "medial" portion of the same nerve shown in Figs. 1, 2 and 3. Note the variations in size of the fibers and the proportion of myelin to axis cylinder. Compare with Figs. 1 and 8. Polarized light method. Section cut at 10 microns.
- FIG. 8. Normal nerve. Microphotograph of "distal" portion of the same nerve shown in Figs. 1, 2, 3 and 4. Note the decrease in size of the fibers to the axis cylinders as compared with "proximal" and "medial" portions. Polarized light method. Section cut at 10 microns.
- FIG. 9. Degenerated nerve. Microphotograph of a nerve which had been transected 72 hours showing the irregular black precipitation seen in Marchi preparations. Compare with Fig. 10. Marchi method. Section cut at 20 microns.
- FIG. 10. Degenerated nerve. Microphotograph of a nerve that had been transected for 72 hours as shown by the polarized light method. Note the clear sheath and axis cylinder changes that are shown. Compare with Fig. 9. Polarized light method. Section cut at 10 microns.
- FIG. 11. Degenerated nerve. Microphotograph of a nerve that had been transected 120 hours. Marchi method. Section cut at 20 microns.
- FIG. 12. Degenerated nerve. Microphotograph of a nerve that had been transected 120 hours. Polarized light method. Section cut at 10 microns.



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PLATE 44

FIG. 13. Normal nerve. Microphotograph of a fresh (unfixed) nerve showing the lack of differentiation and structural detail as compared with the fixed nerve shown in Fig. 1. Such preparations react only minimally to rotation of the stage. Polarized light method. Section cut at 10 microns.

FIG. 14. Normal nerve. Microphotograph of a section cut at 20 microns of the same portion of the same nerve shown in Fig. 13. Note the increase in birefringent material as compared to Fig. 13 and the lack of typical fibrillar detail as shown by fixed nerves. Polarized light method.

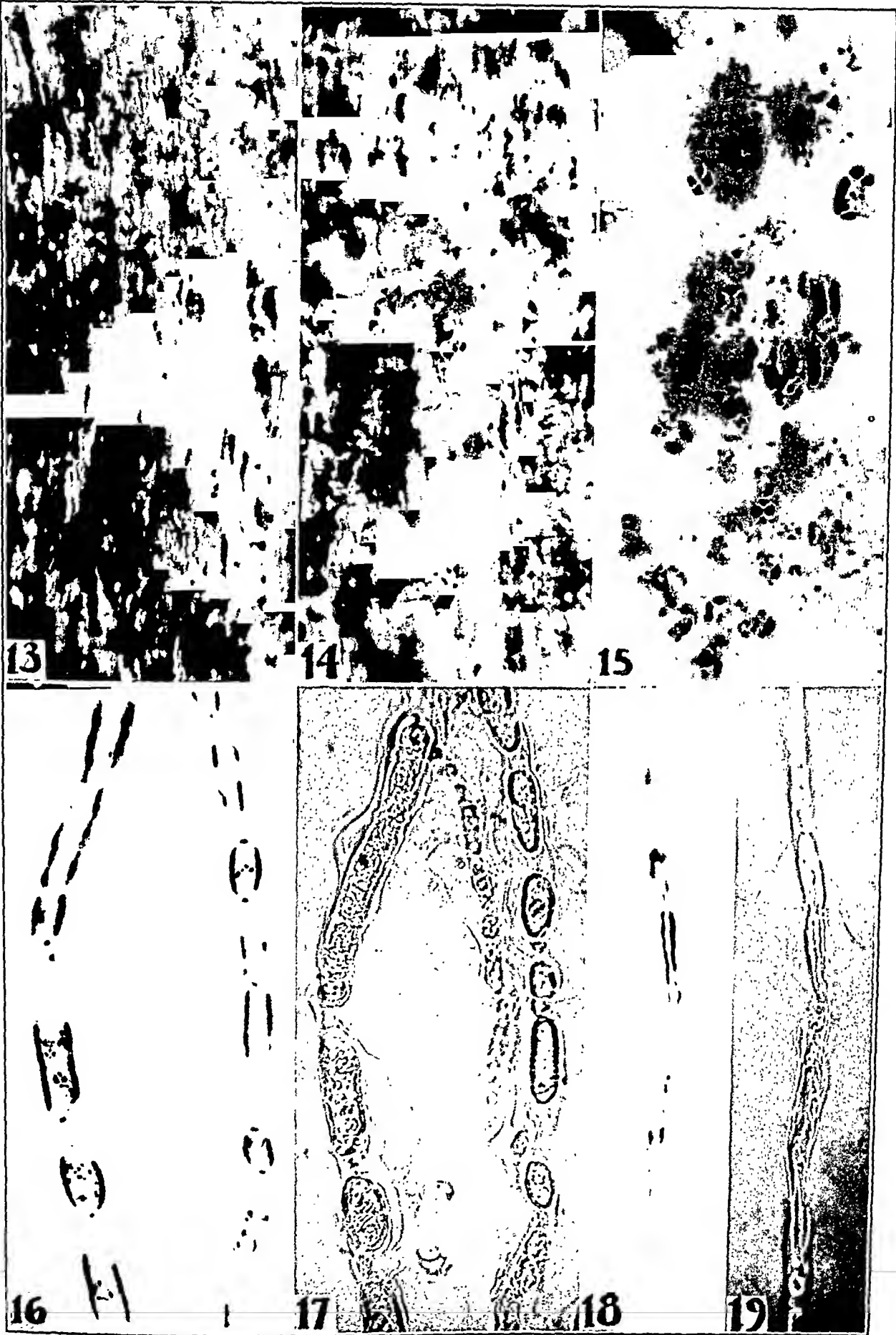
FIG. 15. Degenerated nerve. Microphotograph of a section of a fresh (unfixed) nerve that had been transected for 96 hours. The similarity between the fixed and unfixed nerve is greater than in the normal nerve. Such sections react slightly to rotation of the stage, but structural detail is much less clear and definite than in fixed nerves. Polarized light method. Section cut at 10 microns.

FIG. 16. Degenerated nerve. Microphotograph of isolated fibers from a nerve that had been transected 72 hours. Note the segmentation and the completely isotropic areas between the segments and between the two fibers shown. Compare with Fig. 17. Polarized light method. Section cut at 10 microns.

FIG. 17. Degenerated nerve. Microphotograph of the same portion of the same nerve shown in Fig. 16, showing the advantages of uncrossing the Nicol prisms to determine whether segmentation seen with crossed Nicols is indicative of fragmentation, and also to determine whether a blackened area is a split between fibers or an area of degenerated material that has become completely isotropic. Note the continuity of the sheaths of both fibers and the appearance under these circumstances of an isotropic fiber that had been "masked" by the crossed Nicols. The wavy material between the fibers represents interfibrillar connective tissue. Section cut at 10 microns.

FIG. 18. Degenerated nerve. Microphotograph of an isolated fiber from the same nerve shown in Figs. 16 and 17. Note the difference in size of the fibers in these sections. Compare with Fig. 19. Polarized light method. Section cut at 10 microns.

FIG. 19. Degenerated nerve. Microphotograph of the same portion of the same nerve shown in Fig. 18 with the Nicol prisms uncrossed. Note that the smaller size of the fiber is to a large extent the result of perifibrillar increase in isotropic material. Without examination with uncrossed Nicol prisms this fiber would have been considered merely a smaller or a less edematous fiber than those shown in Fig. 16. Section cut at 10 microns.







# HISTOPATHOLOGY OF THE PERIPHERAL NERVES IN ACUTE AND CHRONIC VITAMIN B<sub>1</sub> DEFICIENCY IN THE RAT \*

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## INTRODUCTION

Some investigators <sup>1-3</sup> have reported that vitamin B<sub>1</sub> deficiency produces myelin degeneration in the peripheral nerves; others <sup>4-8</sup> have failed to find evidence of such changes. A further study was undertaken, therefore, in an effort to find some explanation of the disagreement in results. The study was planned to compare the effects of different diets and different levels of feeding as well as the effect of an acute deficiency with that of a chronic deficiency.

The present paper is concerned only with the results of the studies on the peripheral nerves. The polarized light method was the principal method used, since it has been shown <sup>9, 10</sup> to be more sensitive and more reliable for the detection of early myelin changes than the Marchi or the sudan III methods. The study included, however, observations of preparations made by the use of the sudan III method.

## EXPERIMENTAL

### *Histological Technic*

The histological technic was essentially the same as that described previously.<sup>10</sup> The rats were killed with chloroform and the posterior extremities were fixed in 10 per cent neutral formalin before removal of the nerves. The sciatic nerves were then removed from the legs and stored in fresh fixative until sectioned. Frozen sections 10 microns in thickness were used for both the polarized light and the sudan III methods.

### *The Acute Deficiency*

*Animals and Diets:* This group consisted of 133 animals, 39 of which received the stock diet <sup>4</sup> as a control diet, which was known to be adequate for both growth and reproduction. The diets and

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levels of feeding have been discussed in a previous report.<sup>11</sup> Animals receiving the various types of diets were used, but since the type of diet had no apparent effect on the condition of the nerves, it will be disregarded in this report. The level of feeding, however, did affect the condition of the nerves as will be shown later. There were 63 animals studied from the limited group (food limited to a maximum of 12 calories per rat daily and isocalorically controlled throughout all deficient and control diets); 44 of these received the vitamin B<sub>1</sub> deficient diets, 10 received the deficient diets plus vitamin B<sub>1</sub>,\* and 9 received the stock diet. There were 70 animals studied from the *ad libitum* group; 29 of these received the deficient diets, 11 received the deficient diets plus vitamin B<sub>1</sub>,\* and 30 received the stock diet.

With the exception of 8 animals used to study the effect of a vitamin B<sub>1</sub> deficient diet on the nerves of animals becoming moribund before developing the neuromuscular symptoms, all the deficient animals were allowed to develop severe neuromuscular symptoms before being killed. The average length of time the animals were left on experiment after developing neuromuscular symptoms was 2.5 days. No postmortem material was used for histopathological studies.

*Gross Pathology:* At autopsy the peripheral nerves of animals in the deficient and control groups presented no abnormalities visible in the gross.

*Microscopic Pathology:* The peripheral nerves of the deficient animals, when examined by the polarized light method, showed a variable degree of edematous change that did not appear to be related to the diet, the level of feeding, the time on experiment, or the intensity of the symptoms (Fig. 2). In no case did the edema approach a stage comparable to that seen in nerves transected for 24 hours.<sup>9, 10</sup> All fibers appeared involved and both the myelin sheath and the axis cylinder shared in the enlargement. At times the margins of the axis cylinders were somewhat roughened and the incisures of Schmidt-Lantermann contained more isotropic material than is normally seen. Occasional droplets of isotropic material were found in the myelin sheaths, but these were comparatively infrequent. The nodes of Ranvier were occasionally enlarged and dumb-bell shaped. Sudan III preparations showed

\* Fuller's earth absorbate.<sup>12</sup>

no evidence of this edema or of any degenerative change. Perineural fat, however, was intensely stained.

The peripheral nerves of the control animals that received vitamin B<sub>1</sub> and the limited (isocaloric) amounts of food were significantly different from those of the deficient animals (Fig. 3). In the animals receiving the stock diet, as well as those receiving the experimental diets, a considerable number of the fibers showed changes typical of advanced Wallerian degeneration; the animals that received the stock diet showed the greatest number of degenerating fibers. The nerves of animals receiving the high carbohydrate diets plus vitamin B<sub>1</sub> showed slightly more degeneration than those from animals on the 23 per cent coconut fat diets. The degenerative change apparently was not confined to patchy areas but was seen anywhere in the sections and usually extended throughout the fiber. In addition the edematous changes observed in the deficient group were present in the greater number of animals.

The nerves of the control animals that received vitamin B<sub>1</sub> and food *ad libitum* showed no evidence of degenerative changes in amounts greater than normally present.<sup>12</sup>

In contrast to the above are the nerves of 8 animals in the deficient groups which had shown severe anorexia and progressive weakness and which finally became moribund without developing neuromuscular symptoms. These animals showed more Wallerian degeneration than is normally seen, but less than the controls on the limited (isocaloric) feeding schedule. The edematous changes seen in other rats in the deficient groups as well as in the control groups were also present to a variable extent. Sudan III preparations showed the Wallerian degeneration but no other evidence of abnormality.

### *The Chronic Deficiency*

*Animals and Diets:* This group consisted of 40 rats, all of which received diets deficient in vitamin B<sub>1</sub>. The diets were essentially similar to those used in the study of the acute deficiency.

The animals in this group were first allowed to develop the severe neuromuscular symptoms characteristic of the acute deficiency and were then fed small amounts of thiamin crystals dissolved in distilled water. Administration was by means of a

catheter into the stomach. The dosage was kept as small as possible to prevent complete relief of symptoms, but large enough to keep the animals alive. The dosage varied from 1.5 gamma to 100 gamma depending on the severity of the condition at the time of administration. Usually 1.5 gamma per rat daily was sufficient. The rats in this group were continued on experiment from 3 to 99 days after the development of the initial attack. However, it was not always possible to prevent cures, nor was it possible to prevent deaths even after larger doses of vitamin solution. The animals included in this study, however, had all shown residual symptoms for a considerably longer time than is possible without supplementation with vitamin B<sub>1</sub>.

*Symptomatology:* Partial alleviation of the symptoms promptly occurred after administration of the thiamin chloride, except in a few animals which did not respond even after larger doses were given. The residual symptoms can be roughly grouped into two types. In the first type symptoms of impaired equilibration were manifested by difficulty in maintaining normal posture, by spasmodic and incoordinate movements of the head, deviation of the head to the right or left, or head retraction, and unilateral or bilateral deviation or protrusion of the eyeball. Loss of the vestibular righting reflex and convulsive attacks were also present to varying degrees and were usually continued throughout the experimental period. Such animals, if inadvertently cured by too large a dose of vitamin, showed a return of symptoms after a variable time had elapsed. Usually the attacks became progressively more severe and finally reached a stage where cure was impossible even when large (100 gamma) doses of thiamin chloride were given.

The second type of residual symptoms consisted, in its mildest form, of a peculiar ataxic hesitant type of walk which is not often seen in the acute condition. This condition was often present immediately after the vitamin was fed, but more frequently became apparent only some days after supplementation. More severe cases showed definite partial paralysis of one or both hind legs. Involvement of the forelegs occurred but this was infrequent. In most cases the condition became progressively but slowly worse; in a few animals the condition showed no increase in severity. Finally, a complete flaccid paralysis of one or more extremities

was often seen. Sensation in the affected extremity was impaired but not absent. Pricking with a needle or exerting pressure on the paw produced very little reaction. In some of the more severely affected animals subcutaneous edema of the affected extremity was noted. In fact, these animals showed considerable resemblance to those in which the sciatic nerve had been transected.

The equilibratory and the ataxic or paralytic type of symptoms were often present in the same animal, but they were also seen as separate manifestations in numerous instances.

It is of interest in connection with the supplementation with thiamin chloride to note that a prompt increase in food consumption and in weight occurred. Typical of this are the results obtained when an animal which had shown considerable residual symptomatology was given two 100 gamma doses of thiamin chloride in an attempt to cure the symptoms. This animal promptly increased its food consumption and gained 25 gm. during the succeeding week despite the persistence of the symptoms.

*Gross Pathology:* At autopsy the peripheral nerves of a few animals that had shown severe symptoms were observed to have localized enlargements along their course. The enlargements apparently had no particular site of distribution nor did they occur in the nerves of rats receiving any particular diet. In most of the animals no abnormalities were visible on gross examination.

*Microscopic Pathology:* Peripheral nerves examined by the polarized light method showed a variety of changes that were not present in the nerves of animals killed during the more acute phase of the deficiency. The changes were usually of a focal patchy type but occasionally were found to involve the whole nerve trunk.

In those nerves showing the mildest type of involvement there was a type of edematous change similar to but more severe than that seen in the nerves of animals showing the acute condition (Fig. 4). The myelin sheath, axis cylinder and interfibrillar connective tissue all shared in the edema. By uncrossing the Nicol prisms<sup>10</sup> periaxillar accumulations of isotropic material were observed. The myelin sheaths were frequently hazy but contained a few large droplets of isotropic material. However, in a few cases a definite granular appearance of the myelin was present which suggested the accumulation of minute droplets of isotropic material. The nodes of Ranvier were enlarged and dumb-bell shaped

and there appeared to be an excess of granular bubbly material which remained light at the points of extinction. There was an increase in the amount of isotropic material present in the incisures of Schmidt-Lantermann.

In the severely affected nerves the changes were apparently accentuations of the changes just described. The fibers became more enlarged, the axis cylinders became roughened, and more periaxillar isotropic material was present (Fig. 5). In still more severely affected cases some of the fibers became completely isotropic while others showed marked enlargement and very large bulbous areas along their course. In Figure 7 are seen the remnants of two fibers that are almost completely isotropic, while Figure 8 shows the results of uncrossing the Nicol prisms to determine whether these remnants are really isotropic fibers or merely small fragments resulting from uneven cutting of a fiber. Unless this procedure is resorted to one is unable to determine whether a blackened area seen with crossed Nicol prisms represents a space between fibers or a group of isotropic fibers. Figures 13 and 14 show the most severe type of degenerative change encountered in this study. These changes occurred in an animal that had shown a progressive degree of ataxia and paralysis for 57 days after the development of neuromuscular symptoms. The dumb-bell shaped enlargements seen in Figure 13 indicate that the effect is at the nodes of Ranvier. The marked enlargement will be better appreciated if Figure 13 is compared with Figures 1 and 2.

Nerves prepared by the sudan III method showed no evidence of the degenerative change so clearly and consistently depicted by the preparations examined with polarized light.

### DISCUSSION

It is apparent from the results of this study that the effect of an acute vitamin B<sub>1</sub> deficiency on the peripheral nerves is mild as compared with the effect of a more prolonged or chronic deficiency. In the acute deficiency even the sensitive polarized light method failed to reveal any changes other than a few fibers of Wallerian degeneration, such as were also seen in normal nerves, and a mild edema. The latter likewise occurred in the nerves of control rats receiving adequate vitamin B<sub>1</sub> but whose food intake was limited to that of the deficient rats. This limitation of food intake also

increased the incidence of Wallerian degeneration in the groups receiving vitamin B<sub>1</sub> as compared with similar groups not receiving the vitamin. It is believed that this was the result of a more severe inanition in the rats receiving the vitamin.

The chronic deficiency, however, produced marked changes of an edematous type in the peripheral nerves; the fibers were enlarged and in some cases showed large bulbous areas along their course, and there was a variable increase of isotropic material, some fibers becoming completely isotropic. It seems significant, in view of such marked changes in the nerves, that a stage is eventually reached where the symptoms cannot be cured even by the administration of relatively large doses of thiamin chloride. This, of course, might mean the lack of another essential factor of the vitamin B complex. The fact that the symptoms could be cured by thiamin chloride earlier in the history seems to indicate an eventual irreparable damage to the tissues rather than the lack of an additional factor. Moreover, rats receiving the same basal diets used in this study supplemented with thiamin chloride have shown no abnormal symptoms other than a slightly subnormal rate of growth in an experimental period of 18 weeks.<sup>13</sup> Gildea, Kattwinkel and Castle<sup>14</sup> have also reported that after repeated cures the process became irreversible and vitamin B deficient dogs were no longer cured by large doses of vitamin B concentrate.

### SUMMARY

1. The histopathology of the peripheral nerves of rats showing either acute or chronic vitamin B<sub>1</sub> deficiency has been studied by means of the polarized light and sudan III methods.
2. The nerves of rats that had developed symptoms of the acute deficiency showed only a few fibers of Wallerian degeneration, such as may be seen in normal nerves, and a mild edema.
3. The nerves of control rats receiving adequate vitamin B<sub>1</sub> but whose food intake was limited to that of the deficient rats likewise showed a mild edema and more Wallerian degeneration than was found in the nerves of the deficient rats.
4. Nerves of deficient rats that became moribund without developing typical neuromuscular symptoms showed more Wallerian degeneration than the nerves of those that developed neuro-

muscular symptoms, but less than the control rats receiving adequate vitamin B<sub>1</sub> with a limited food intake.

5. Residual symptoms consisting of disordered equilibration or paralysis occurred in rats in which a state of chronic deficiency had been produced by the administration of small amounts of thiamin chloride after the development of neuromuscular symptoms.

6. The chronic deficiency produced marked changes of an edematous type in the peripheral nerves. The fibers were enlarged, in some cases showing large bulbous areas along their course, and contained increased amounts of isotropic material; in the most severe cases some fibers were completely isotropic. These changes were demonstrable by the polarized light method but not by the sudan III method.

7. The rats eventually reached a stage where cures were no longer possible even by the administration of relatively large doses of thiamin chloride. The changes in the nerves indicated that this was the result of irreparable damage to the tissues.

Note: The authors wish to thank Miss Cornelia Stevens for aid in the preparation of the tissues and the microphotographs.

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## DESCRIPTION OF PLATES

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### PLATE 45

All photographs except Figure 8 were taken between crossed Nicols at the point of greatest birefringence at a magnification of  $\times 500$ . Wratten metallographic plates and a contrast developer (Eastman D-19) were used. All sections were cut at 10 microns.

FIG. 1. Normal nerve. Showing the typical structure of normal fibers as shown by sections 10 microns in thickness. The darkened portions of the nerves have been shown by observation with uncrossed Nicols to be the results of tangential cutting and not to increased isotropic material.

FIG. 2. Acute deficiency. Showing the increased size of the fibers, the increase in isotropic material at the incisures of Schmidt-Lantermann, and occasional droplets of isotropic material in the myelin sheath. The borders of the axis cylinders are more ragged than normal and observation with Nicols uncrossed shows slight periaxillar accumulations of isotropic material at times.

FIG. 3. Control animal receiving limited (isocaloric) amounts of food plus ample vitamin B<sub>1</sub>. Showing one of the numerous fibers in advanced stages of Wallerian degeneration. Note also the haziness and darkening of the myelin sheaths. In the upper left corner is a fiber showing an earlier type of change.

FIG. 4. Chronic deficiency. This type of change is common in the nerves of animals that have shown residual ataxia or symptoms of disordered equilibration. Note the increase in size of the fibers, the ragged axis cylinders, and enlargement of nodal structures.

FIGS. 5 and 6. Chronic deficiency. Showing a progressive increase in the severity of the myelin and axis cylinder changes in the nerves of animals showing definite paralysis.

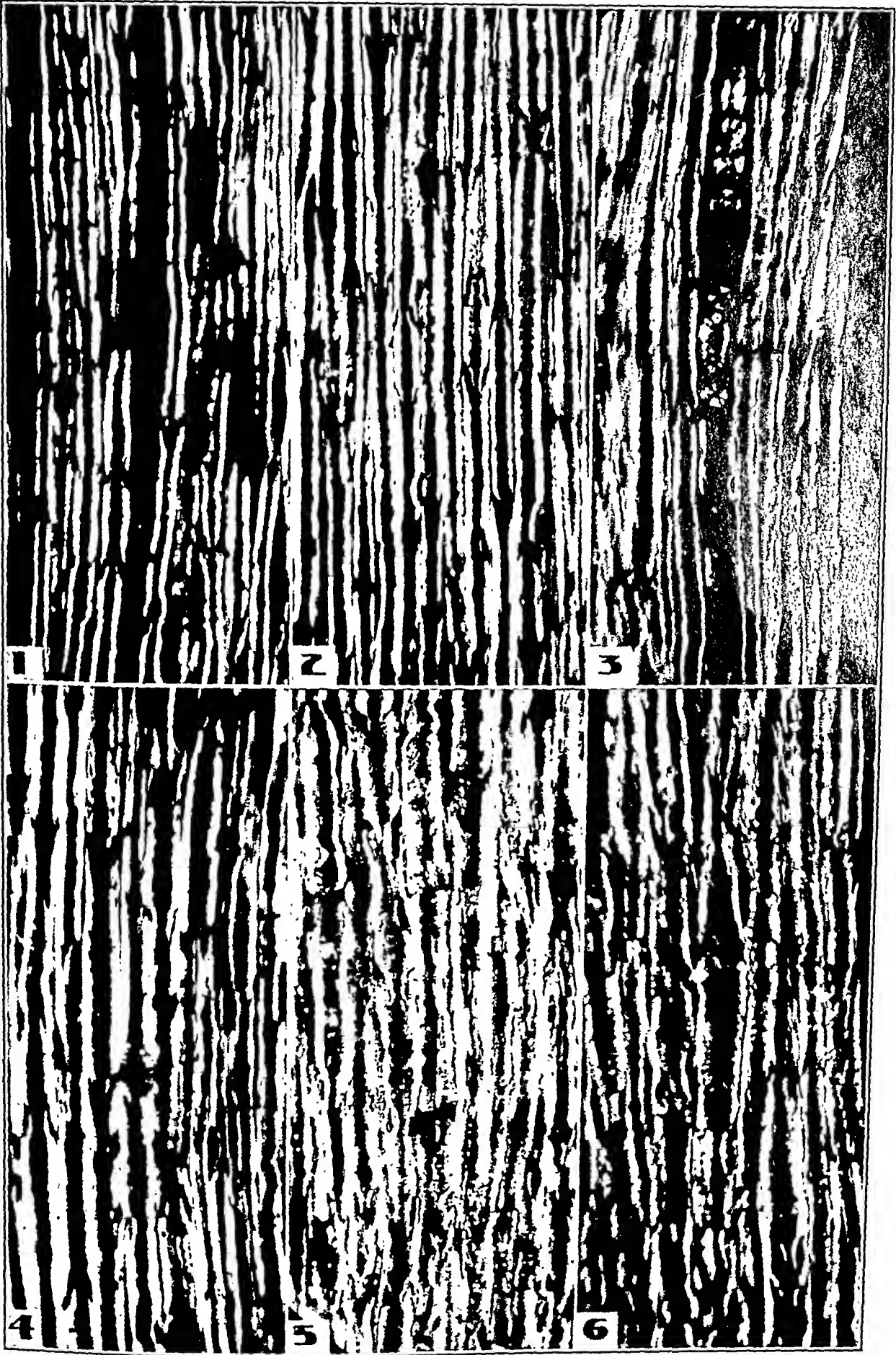


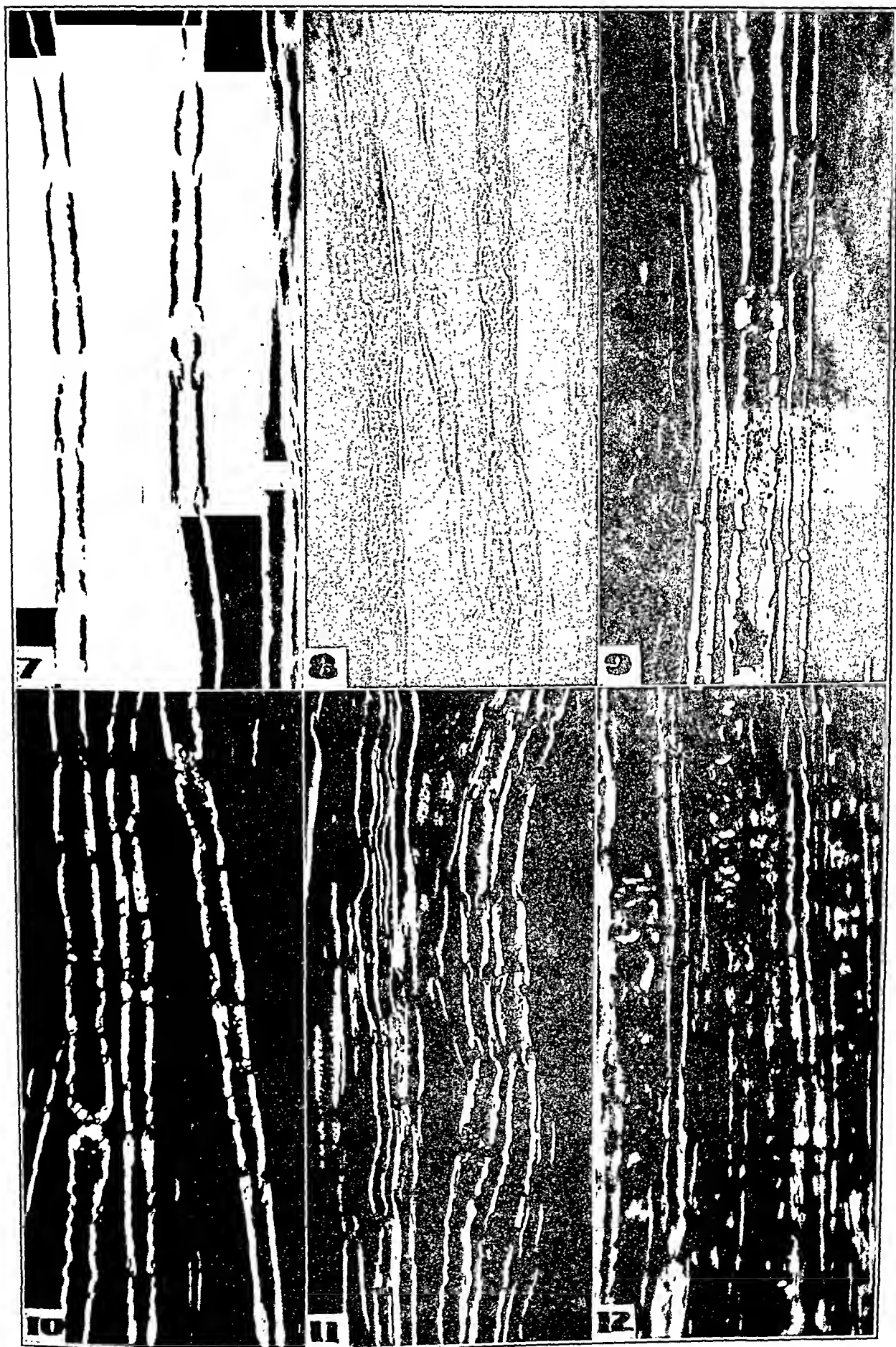
PLATE 46

FIG. 7. Chronic deficiency. Isolated fibers showing a moderately severe degree of change. The remnants of two fibers which are almost completely isotropic are seen. Compare with Fig. 8.

FIG. 8. Chronic deficiency. Photograph of the same fibers as shown in Fig. 7, taken with Nicols uncrossed. Note that the fibers seen as remnants in Fig. 7 are really still present within their sheaths. The wavy material represents interfibrillar connective tissue.

FIGS. 9-11. Chronic deficiency. Isolated fibers showing more severe changes in the size of fibers and presence of isotropic fibers. Note the nodal enlargements.

FIG. 12. Nerve from a chicken which had shown typical symptoms of neurolymphomatosis gallinarum, showing changes typical of advanced Wallerian degeneration.



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Acute and Chronic Vitamin B<sub>1</sub> Deficiency

PLATE 47

FIG. 13. Chronic deficiency. Nerve showing a very severe type of edematous change. Note the marked enlargement of the fibers, particularly at the nodes, the presence of numerous fibers which are almost completely isotropic, and the focal nature of the changes.

FIG. 14. Chronic deficiency. Showing bulbous enlargement along the course of the fibers in nerves which are severely affected. Compare the size of the fibers with Figs. 1 and 2.







# THE RESPONSE OF THE CENTRAL NERVOUS SYSTEM TO THE APPLICATION OF CARCINOGENIC HYDROCARBONS \*

## I. DIBENZANTHRACENE

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The possibility of producing tumors of the brain in experimental animals by the application of carcinogenic substances may be dated from the discovery in 1932 by Burrows, Hieger and Kennaway of the carcinogenic properties of 1:2:5:6 dibenzanthracene. Previous to this time considerable work on the carcinogenic properties of coal tar fractions had been accomplished. However, because of the high non-specific toxicity of such fractions, their application in almost all experiments was necessarily limited to the enduring skin. In this crude tar era there appears to have been only one attempt recorded<sup>1</sup> to apply such material to the brain of an experimental animal. The investigator injected an emulsion of tar into the subarachnoid space of rabbits by suboccipital puncture. It is alleged that the production of tar cancer on the ear and in the stomach was thereby accelerated. No mention is made of how the brain of the rabbit reacted to this treatment.

The introduction of dibenzanthracene, followed soon by a series of polycyclic hydrocarbons of high carcinogenic potency, immediately widened the scope of experimental cancer study. These chemicals, though exceedingly potent, are generally almost non-toxic, so that their application to internal organs and tissues becomes only a matter of interest and operative technique. Many experimenters were soon at work on such projects, and the diversity of tissues tested and results obtained may be found conveniently summarized in the comprehensive reviews by Cook and his colleagues.<sup>2,3</sup>

The brain would seem to afford a particularly favorable organ in which to study chemical carcinogenesis. Here there are located

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in close juxtaposition a number of types of tissues of at least two embryological derivations. For their precise recognition there are available a number of excellent specific staining methods. This might be expected to make possible an accurate determination of the rôle of each of the various cell types entering into the formation of neoplasms. Observation of the common laboratory animals in large numbers for many years has shown that spontaneous brain tumors are practically non-existent, so that any positive results produced are immediately significant. Finally, brain tumors form a considerable and important division of human neoplasms; hence any anatomical or physiological information collected by experimental means might prove welcome and useful.

Judging from published reports, there has to date been little interest in attempting experimental carcinogenesis in the brain. In the course of a larger experiment, Ilfeld <sup>4</sup> in 1936 reported having inserted a pellet of cholesterol containing 5 per cent dibenzanthracene into the brain cortex of 2 stock mice. No results were obtained at the end of 8 months, but the number of animals used was manifestly too small for significance to be attached to negative findings.

Oberling, Guerin and Guerin <sup>5</sup> implanted crystals of benzpyrene beneath the pia and into the brains of 10 rats. No local reaction was seen, though only 3 animals survived the 10 months duration of the experiment. All these 3 exhibited pituitary adenomas — one each of chromophobe, spongiocyte, and castration cell type. One additional rat injected intracerebrally with a drop of 0.1 per cent benzpyrene in oil died in the 8th month with a tumor of epitheliomatous type invading the anterior lobe of the pituitary. No direct causal connection was clearly apparent in any case between the chemical applied and the tumor observed.

Athias <sup>6</sup> is apparently at present engaged in observing a series of 33 guinea pigs which he has injected intracerebrally with methylcholanthrene in arachis oil. He reported the occurrence in 1 animal of a "polymorphous cell sarcoma" in the auricle of the heart, but from his description the mass seems to be more likely only an organizing mural thrombus. He gave no results attributable to the action of the chemical in the brain.

Most recently Weil <sup>7</sup> has recorded an experiment on a small series of rats using dibenzanthracene, methylcholanthrene and

styryl 430. No tumors were obtained after 219 days in 3 rats injected with methylcholanthrene. Dibenzanthracene was implanted in 8 rats. Seven of these showed no neoplastic reaction after 229 days. In the 8th there was found a cystic mass lined by stratified squamous epithelium, located in the midbrain and apparently arising from the base of the skull. A direct causal relation between this mass and the implanted chemical was not demonstrated.

A compound, styryl 430, was injected into the brains of 5 rats. Two died soon afterward of injury or intercurrent disease. The remaining 3 survived up to 180 days. In all the latter there were found small cellular proliferations about particles of the chemical in the meninges and brain. While such aggregations undoubtedly contained individual cells resembling tumor cells, the appearance of the masses as a whole is more like an irritation hyperplasia than a true tumor. The masses were very small, there was no expansile or invasive growth, and no proliferation of blood vessels. This reaction falls far short of the aggressive malignant neoplasms which the carcinogenic chemicals are capable of producing in other tissues.

### TECHNIQUE

Following the earlier reported experiments, an attempt was first made to inject the dibenzanthracene in a 5 per cent suspension in lard. This material, however, proved too difficult to manage in the very restricted space available within the cranial cavity of the mouse. It was found on trial almost impossible to inject a small but accurately measured dose with a tuberculin syringe. The material welled out of the trephine wound into the subcutaneous tissues in a most undesirable fashion. Finally, in the few animals successfully injected, the lard vehicle produced a very confusing non-specific granulomatous lesion.

A solution to the problem of the vehicle of injection was furnished by Shear's studies.<sup>8</sup> He found that dibenzanthracene was readily soluble in molten cholesterol, and that pellets of 5 per cent concentration were fully as potent for carcinogenesis as the lard suspensions previously used. They possessed the further important advantage that they were not absorbed and dispersed, but remained recognizable in sections to mark accurately the

point of application of the hydrocarbon. Pellets were therefore prepared by drawing up a molten 5 per cent solution of dibenzanthracene in cholesterol into 1 mm. capillary glass tubes, oiled to facilitate removal of the solidified pellet. The finished pellet was a cylinder approximately 2 by 1 mm., of an average weight of 0.175 mg. and containing therefore about 0.072 mg. of dibenzanthracene.

It was at first hoped that for convenience anesthesia could be produced by an intraperitoneal injection of one of the drugs of the amytal group. This soon proved unsatisfactory because the depth of narcosis could not be controlled. The anesthesia was either so light that the animal struggled, or so deep that there was an unduly large operative mortality.

The technique of operation as finally developed was as follows: Anesthesia was induced by dropping the mouse into a jar containing a wad of cotton saturated with ether. As soon as the mouse lost consciousness, it was removed and placed on the operating table. This table consisted of an 8 inch square of heavy plank, to the center of which was attached a small L-shaped block of suitable size and height to support the animal's head. Anesthesia was maintained with ether on cotton in a small sputum bottle. The hair over the top of the head was roughly clipped, and the skin swabbed first with tincture of iodine and then with alcohol. The scalp was picked up with forceps, and with scissors an incision about 1 cm. long was made in the midline. The scalp was then pressed aside and the skull exposed by scratching away the galea aponeurotica with a dissecting needle. It is important to bare the skull thus, otherwise the soft tissues are caught by the burr with disastrous results. The skull was held firmly against the head block and a hole was cut in the parietal region by means of a 2 mm. spherical burr driven by an electric dental engine. There was usually little bleeding if the large vessel just behind the orbit was avoided.

Before commencing the operation the cholesterol pellets were placed in a small dish of 80 per cent alcohol. One was selected and placed in the tip of a small glass pipette 3 cm. long. The pellet was readily held in position by the capillary attraction of a small amount of the alcohol. The tip of the pipette was placed on the trephine hole, and the pellet pushed down into the brain by

means of a dissecting needle just long enough to expel the pellet from the pipette. The pipette was then laid aside, and the pellet adjusted with the needle so that its superficial end just appeared in the trephine opening. The scalp was drawn back into place and the incision tacked together with a single suture. The wound was finally dressed with a small amount of flexible collodion.

The above operative technique proved both rapid and efficient, and after a little practice the immediate mortality was less than 5 per cent. The animals recovered promptly from the operation and showed no apparent neurological defect. The dressings were usually clawed off in a week or 10 days, healing was sound and the hair was quickly restored. The animals were thereafter fed a good full diet and observed closely. Any animal appearing to be ill was isolated, because cannibalism was common and the eating usually began with the head.

Every precaution was taken to obtain ideal tissue for microscopic study. An animal appearing to be seriously ill was killed with illuminating gas, and the brain at once removed and placed in fixative. Autopsy technique was also the result of considerable experimentation during which some of the earlier brains were partially destroyed. The routine finally evolved was to pin the animal ventral side down and reflect the scalp by a midline incision. The operative field was inspected externally and the pellet identified. Usually the trephine hole was covered by a fibrous membrane through which the end of the pellet could be seen. The vertebral column was cut through with scissors as close as possible to the occiput. The occiput was then lifted up, and one blade of a small pointed scissors was thrust into the foramen magnum. Keeping close to the base of the skull the parietal bones were cut on both sides. A third cut across the frontal bones freed the calvarium. With a little care the whole brain and calvarium with the pellet undisturbed *in situ* could then be lifted away.

Without further manipulation the calvarium with brain attached was placed in Zenker's fluid containing 10 per cent of glacial acetic acid. Fixation for 24 hours in this solution not only afforded perfect tissue preservation but also completely decalcified the skull. After washing, the calvarium and brain were trimmed to a transverse slice 2 mm. thick containing the operative field and the pellet. This slice was embedded in paraffin in the

usual manner. Sections were routinely stained with hematoxylin-eosin, phosphotungstic acid hematoxylin, Masson's trichome stain, and Foot's modification of the impregnation method of Del Río-Hortega for reticulum. A few brains in which the pellet lay deeper and could be detached from the skull were fixed in formalin-ammonium bromide. It was found that frozen sections including the pellet could readily be cut, and specific impregnations such as the gold chloride-sublimate method applied to them. This technique will be employed more extensively in the cytological study to be published later.

### EXPERIMENTAL DATA

Using the operative technique described above, cholesterol pellets were successfully implanted in 81 stock albino mice, and 1 was injected with approximately 0.1 cc. of a 5 per cent lard suspension. The survival times and the number of living mice remaining at the end of each month are shown in Table I.

TABLE I

Time after operation	Number surviving	Time after operation	Number surviving
—	82	180 days	53
30 days	75	210 "	51
60 "	72	240 "	48
90 "	63	270 "	44
120 "	58	300 "	8
150 "	55	330 "	2

Forty-eight animals survived the period of 8 months found by Ilfeld<sup>4</sup> to be the average time required for the production of kidney tumors. Fifty-three lived for more than 6 months, the average interval calculated by Fieser<sup>9</sup> to be necessary for the formation of subcutaneous sarcomas.

In spite of the fairly severe trauma and the possible irritative action of the pellets, only 2 animals exhibited any recognizable neurological signs. These animals, dying 7 and 21 days after operation, showed contralateral extensor spasm and generalized convulsions of less than 24 hours duration. Most of the naturally occurring deaths seemed to be the result of pneumonia or gastroenteritis, and later of senility.

The implanted pellet was definitely identified at autopsy in all

but 8 mice. In the case of 5 of these the heads had been partly eaten and destroyed by cage mates. In the remaining 3 careful search failed to disclose any trace of the pellet. Probably it had been extruded during healing of the operative incision. Usually only the heads of dead animals were examined. A complete autopsy was done in only a few instances where the animal's condition suggested the presence of some interesting visceral disease.

It is not at present possible to describe in detail the sequence of tissue reactions to the implantation of the carcinogenic hydrocarbon. All effort in this first experiment was directed toward keeping as many animals as possible alive through the average incubation period of 8 months. Tissue examination was therefore limited to those animals dying naturally. Since such deaths occurred at irregular intervals, and since some of the earlier material was lost in the search for a suitable technique, the knowledge of tissue changes is very uneven. It is planned to fill in these gaps in subsequent experiments.

What was learned of the tissue reactions in this series was essentially as follows: Introduction of the pellet produced a laceration of the brain substance of considerable severity. More or less hemorrhage occurred about the pellet, and there was a prompt mobilization of microglia. These cells actively phagocytosed and removed the red blood cells and lipoid debris. This scavenger phase apparently lasted about 30 days unless prolonged by infection. Afterward there was left a residual small number of microglia distended with vacuoles of lipoid material. These remained up to the end of the experiment nearly a year later. A few microglia also assumed bizarre and elongated shapes and insinuated themselves into clefts between the crystals forming the pellet.

As early as 20 days after operation there could be demonstrated by silver impregnation a partial sheath of reticular mesodermal fibrils surrounding the pellet. The completeness and density of this sheath varied greatly in different specimens. A small portion of this mesodermal tissue proliferated from the vascular adventitia. The most of it was derived from the dura. The activity of the dura in this process was quite analogous to its well known activity in encapsulating hemorrhages. The pia and arachnoid seemed to react only feebly, if at all. In successful preparations

they could be seen to have been pushed down into the wound between the cortex and the encapsulated pellet with no apparent proliferation (Fig. 1). As expected, the densest encapsulation occurred in those specimens in which the pellet remained in contact with the dura. This was not invariably the case, though, and the degree of encapsulation was so capricious that it must be determined probably by the reactivity of the individual animal. The presence of such a capsule does not prevent the development of tumors. In fact, in the one tumor produced in this experiment, the capsule about the pellet was unusually dense and complete.

Reaction in the fixed nervous tissue was disappointingly mild during the interval of this experiment. Sometimes the ganglion cells receded slightly from the vicinity of the pellet. Gliosis was never marked, and less than one-half of the animals showed more than a minimum glial response. After about 4 months there were usually a few hypertrophied astrocytes with stout fibrils near the pellet. The glial reaction was always more pronounced in the white matter, and its degree was apparently not influenced by the extent of the mesodermal capsule about the pellet. In several specimens the pellet touched upon or penetrated through the ependyma of the lateral ventricle. In none of these was there any appearance of activity in the ependyma. It seemed merely mechanically stretched or displaced. When ruptured it occasionally everted and extended for a short distance outward along the surface of the pellet (Fig. 2). In a few animals portions of the choroid plexus lay in contact with the pellet but exhibited not the slightest morphological alteration. In these instances of contact or penetration of the pellet into the ventricle the cavity was often moderately dilated, but no explanation for this appeared in sections.

The reaction of the pia-arachnoid was correspondingly feeble. Usually it appeared merely stretched and ruptured by the pellet. In 8 specimens of 8 to 9 months duration there appeared small cellular thickenings of the arachnoid near the lips of the cortical wound, but not in direct contact with the pellet (Fig. 3). In minute structure such cell groups resembled the human meningioma, but they were exceedingly small and they showed no evidence of active proliferation or of pressure on adjacent tissues.



Accordingly for the present they do not seem to merit classification as true neoplasms.

Considering the crudity of sterile operative technique, there was relatively little evidence of serious meningeal infection observed in this series of animals. A chronic type of meningo-encephalitis, consisting of meningeal and perivascular round cell infiltrations with occasional focal parenchymatous lesions, was observed in 16 animals. In 6 of these such changes were slight, in 6 moderate, and in 4 marked. Since all degrees of this inflammatory lesion were observed to occur quite at random in mice dying at all stages of the experiment, it seems that the health of the animals was not seriously affected thereby. In only 1 animal a mixed fungus infection produced an abscess about the pellet and caused death on the 21st day.

The skull exhibited practically no reaction to the presence of the pellet. The trephine hole was closed by a dense acellular collagenous membrane derived indistinguishably from the dura and the periosteum. In the great majority of animals there seemed to have been little or no new bone formation and in no instance was bony healing complete. In some, the presence of one end of the pellet in contact with the trephine wound probably inhibited new bone formation. This mechanism, however, fails to explain the lack of bony healing in the fairly numerous animals in which the pellet was completely buried in the brain. In 1 animal killed 326 days after operation the entire calvarium was found to be increased to three times normal thickness. The bone showed a microscopic structure somewhat resembling that of Paget's osteitis in man. There was also partial ossification of the fascia covering the temporal muscles, and a lesser amount in the capsule surrounding the pellet. No evidence of inflammation appeared in adjacent tissues. As the condition was not recognized at the time of killing the animal, only the head was examined.

In this experiment only one tumor was directly caused by the carcinogenic chemical. This appeared in a male mouse found dead 282 days after operation. The tumor consisted of a moderately soft, hemispherical gray plaque of tissue 8 by 6 by 3 mm. firmly attached to the external surface of the skull over the point of inoculation. The scalp was intact and freely moveable over the tumor mass. Sections showed that the pellet lay obliquely

tangential, in contact with the brain, skull and periosteum. The pellet was apparently completely encapsulated, but tumor tissue had nearly destroyed the external half of the capsule. The tumor appeared to well up from the surface of the pellet through the trephine opening and spread over the skull beneath the scalp (Fig. 5). The outer table of the skull was partly eroded, and tumor tissue was beginning to invade the diploe. There was no definite intracranial extension, but for a short distance on either side of the trephine hole the dura was loose, cellular and infiltrated with a moderate number of round cells.

Histologically the tumor consisted of interlacing bundles of plump anaplastic spindle cells showing occasional mitoses (Fig. 4). It closely resembled the already familiar type of spindle cell sarcoma readily produced by the subcutaneous injection of dibenzanthracene. The presence in some fields of long, multinucleated, strap-like giant cells suggested a muscle cell origin. However, the definitely striated cells occurring in the tumor were clearly fragments of the partly destroyed occipitofrontalis muscle. By means of silver impregnation, reticulum could be seen to form a sheath about many tumor cells similar to that about muscle fibers. The tissue was unfortunately not sufficiently well preserved to permit the demonstration of myoglia fibrils. Blood vessels appeared as small thin-walled capillaries, or in the center of the mass more often in the form of irregular clefts between the bundles of cells. No hemorrhage or necrosis was seen. Small foci of lymphocytes were sparsely scattered in the tumor mass, and larger numbers were present along the advancing edge next the skull. The viscera showed no trace of metastases. Attached to the wall of the right auricle was an organized mural thrombus.

In this series of 82 animals 4 incidental neoplastic conditions were observed. None could be attributed to the experimental treatment. Two were the familiar type of spontaneous breast carcinoma, recognized 38 and 272 days after operation. A third tumor appeared as a large, rather soft and cellular mass beneath the angle of the jaw on the left in a male mouse 268 days after operation. Histologically this tumor was a rapidly growing small alveolar carcinoma, interpreted as arising probably from the thyroid. No regional or distant visceral metastases were found in any of these animals. The last neoplastic condition encountered

was an acute leukemia, which appeared in a male mouse 242 days after operation. Gradual enlargement of the abdomen had been noticed for 2 weeks before death, and the animal was carelessly assumed to be pregnant. It was found later moribund and killed, and the abdominal distention was discovered to be due to an enormously enlarged liver mottled with small yellowish patches of necrosis. Microscopically the liver, spleen, kidneys, adrenals and lymph nodes presented a massive leukemic infiltration. The heart and the vessels in the brain and other organs contained large numbers of immature leukocytes. These cells were large and non-granular, with round or indented vesicular nuclei, and closely resembled the blood monocyte. Unfortunately no blood or bone marrow studies were made because the nature of the condition was not recognized at the time of autopsy.

#### SUMMARY AND CONCLUSIONS

Cholesterol pellets containing 5 per cent of 1:2:5:6 dibenzanthracene were implanted into the brains of a series of 81 stock albino mice. The operative and histological technique employed has been described in detail.

Fifty-three of these 81 animals survived over a period of 6 months, the average interval shown by Fieser to be required for the production of subcutaneous sarcoma by means of dibenzanthracene. Histological observations indicated that the introduction of the pellets produced brain laceration followed by a marked phagocytic microglial response which disposed of the debris in about 30 days. A partial to complete collagenous capsule, chiefly from the dura, formed about the pellet. Thereafter a state of equilibrium between tissue and pellet seemed to be established.

On the 282nd day an extracranial, malignant spindle cell tumor was found, originating about the superficial surface of a pellet. In all, 40 animals survived longer than the period required to produce this tumor and were killed during the ensuing fortnight to end the experiment. In none of these did there appear any definitely neoplastic tissue reaction.

In the course of this experiment 4 spontaneous neoplastic conditions, 3 carcinomas and a leukemia, were encountered.

A dose of 1:2:5:6 dibenzanthracene known to be sufficient in

amount and duration of action regularly to produce subcutaneous sarcomas was applied to the brain and meninges. In this experiment one such sarcoma was produced about the exposed end of a pellet, but no neoplastic reaction was seen in the brains or meninges. It is therefore concluded that the brain and meninges of the mouse respond only very slowly or not at all to the carcinogenic stimulus of 1:2:5:6 dibenzanthracene.

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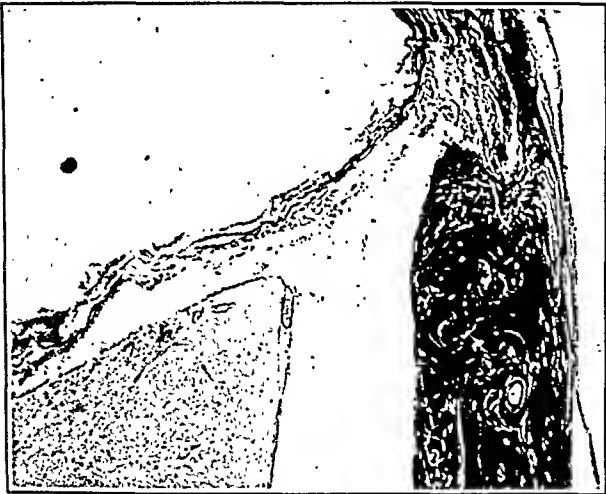


## DESCRIPTION OF PLATE

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### PLATE 48

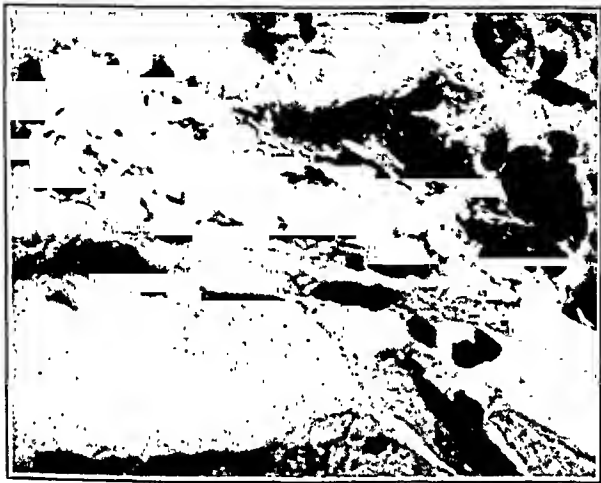
- FIG. 1. Encapsulation of pellet by fibrous tissue proliferating from the dura and periosteum closing the trephine hole. Below this and quite separate, the delicate pia arachnoid covers the brain surface and passes down into the cortical wound. The pellet, dissolved during embedding, occupied the left upper third of the field. Foot-Hortega impregnation.
- FIG. 2. Penetration of the pellet into the lateral ventricle with eversion of the ependyma. Phosphotungstic acid hematoxylin stain.
- FIG. 3. Focal proliferation of cells on the external surface of the arachnoid in the vicinity of the pellet. Note the whorl formation on the right. Hematoxylin-eosin stain.
- FIG. 4. Histological structure of the extracranial sarcoma shown in Figure 5. Two mitoses appear in the left center. Hematoxylin-eosin stain.
- FIG. 5. Transverse section of brain and calvarium showing tumor mass arising from the external surface of the encapsulated pellet and spreading over the surface of the skull. To the right of the pellet the external table has been broken through and tumor is beginning to invade the diploe. In the right edge of the tumor are strands of the occipitofrontalis muscle. Masson's trichrome stain.



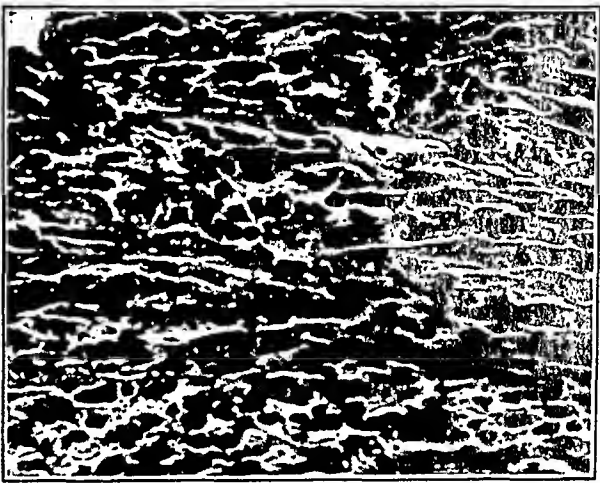
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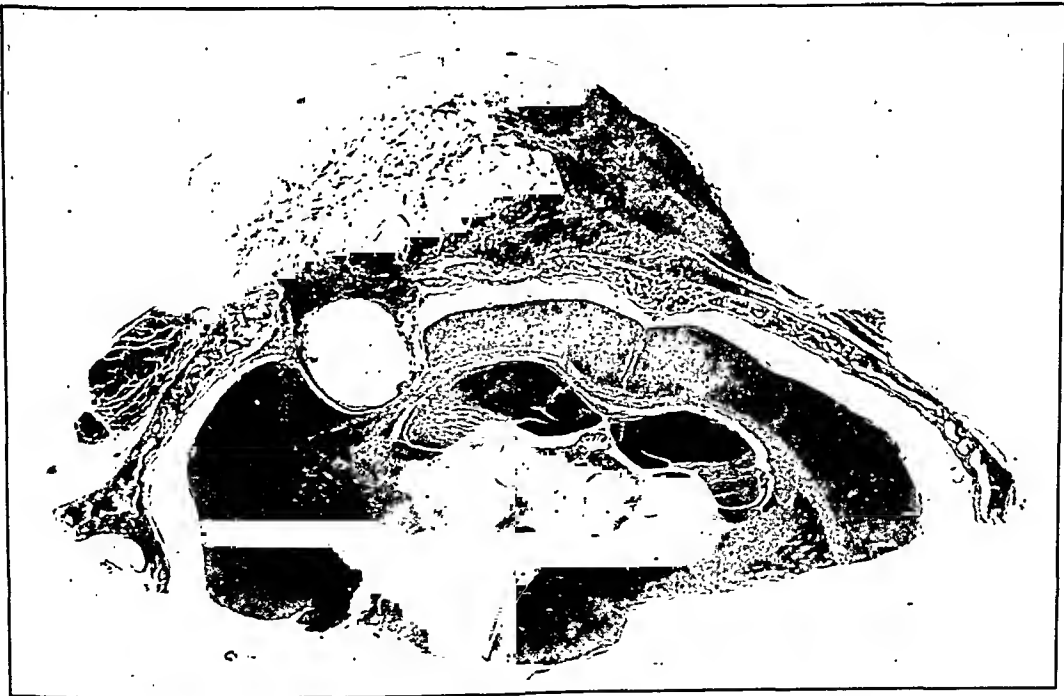
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Peers

Response of Central Nervous System





# A STUDY OF NORMAL AND RACHITIC BONE STRUCTURE BY MICROPHOTOGRAPHIC METHODS \*

LOUIS SIEGEL, ROY M. ALLEN, GRACE MCGUIRE AND K. GEORGE FALK

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Laboratory of Roy M. Allen, Bloomfield, N. J.)*

## INTRODUCTION

Since the discovery that vitamin D can prevent and cure rickets considerable work has been done on the mechanism involved and numerous studies of normal and rachitic bones by roentgenograms, silver impregnation methods, calcium estimations, and so on, have appeared in the literature. Microphotography has aided materially in these studies, not only by revealing structures visible through the microscope, but because of the sensitivity of plates to wave lengths beyond visible range, further structural and other details are also revealed. Recent improvements in photosensitive materials have furnished new and better instruments for the study of detailed structures. A series of microphotographs based on the radiation of different wave lengths (ultraviolet, normal (green), and infrared) on unstained bone sections are presented in this paper, illustrating how the penetrative powers of the wave bands employed affect the photographic plates differently.

## EXPERIMENTAL METHODS

Normal, rachitic, and partially healed rachitic bones from rats were obtained by experiments carried out in the bioassay of the vitamin D content of foods. The method of assay was the U. S. P. XI, the Steenbock rickets-producing ration No. 2965 being used, and the extent of the rickets was determined by the line test on the corresponding bones of the same animals. The normal bones were from rats fed a normal diet and of the same age (55 to 60 days) as the rats with rickets. The femurs of normal, 2 plus healed (complete healing being represented by 4 plus), and rachitic rats were sectioned without preliminary decalcification. They were first embedded in celloidin and then cut in sections

\* Received for publication October 5, 1938.

25 microns thick. The sections were mounted in glycerin under fused quartz coverslips on fused quartz microscope slides. The objective, eyepiece and substage prism of the microscope, as well as the slide and the coverslip of the preparation, were all of quartz, since ultraviolet light, to which ordinary glass is opaque, was one of the forms of radiation used. Even the walls of the cooling cell and the light filter were of quartz. An open automatic arc was used as the source of light in all the illustrations.

Since the object of this study was to compare the effect of ultraviolet, normal (green), and infrared wave bands on bone sections, the same optical system was used in all the microphotographs. All were taken at 18 diameters magnification.

To ensure negatives of comparable density, despite the different plates and filters necessary, the exposures required were worked out by the multiplier method. This consists of taking a series of graduated exposures of strip photographs on the same plate. Leaving the light on continuously, the slide of the plateholder was only partially ( $\frac{1}{2}$  inch) withdrawn and the shutter opened for an extremely short exposure. The slide was then opened to its next  $\frac{1}{2}$  inch strip and an additional short exposure made. This process was continued until a series of strip exposures varying from certain underexposure to certain overexposure was obtained. By changing the filter and plate, a strip exposure plate for the next series of photographs was prepared. In all, three such exposure plates were produced, one for each wave band employed. All were then tank developed simultaneously by the time and temperature method. Examination of the plates revealed sections correctly exposed and of comparable density. In all subsequent work these comparable exposures were used.

The microphotographs will be presented in groups of three for each bone section. Using the exposure data obtained in the strip multiplier plates, three microphotographs in each group were made of the same section without removal from the microscope. The ultraviolet exposure was taken on a Hammer slow plate using a Schott and Gen. filter No. U. G. 2, which transmits light in the 3000 to 4000 Ångstroms wave band. The normal exposure was made on a Wratten "M" plate with a Wratten B2 filter, which passes light in the 4800 to 6000 Ångstroms wave band. The infrared was taken on an Eastman spectroscopic infrared plate 1P

with a Schott and Gen. filter No. R. G. 8, which transmits light in the 7300 to 9300 Ångstroms wave band.

In making the prints, all received the same exposure in the printing machine, and for each group the same time and temperature development was used. Thus, these microphotographs are as nearly comparable as it appears possible to make them under the given conditions.

### EXPERIMENTAL RESULTS

In Figures 1-9 are shown three groups of microphotographs of femurs of normal rats (Figs. 1-3), partially healed (2 plus) rats (Figs. 4-6), and rachitic rats (Figs. 7-9).

Examination of the three figures in each group reveals in each case a progressive clarity of detail and fineness of structure, especially in the epiphyseal layers, in progressing from the ultraviolet plate, with its excessive contrast, through the green to the infrared.

Dr. Clay R. Murray, associate professor of surgery at the College of Physicians and Surgeons, Columbia University, New York City, examined these microphotographs carefully. The following statement by him is presented with his permission:

"In examining the photographs so taken, both with and without the aid of a lens, it is readily evident that the definition of gross differences in the epiphyseal and para-epiphyseal regions, and in the epiphyseal plate in the three groups — normal, healing rachitic and frank rachitic — is much more marked in the infrared exposures than in either the normal or the ultraviolet exposures. The ultraviolet are less satisfactory than the normal (green band). In addition, there is discernible, it seems to me, in the infrared pictures a far greater number of structural and architectural details than in either of the other types of exposure, or in the bone viewed with normal light and a lens."

In order to determine whether the density of the ultraviolet prints obscured detail or not, a series of lighter prints was made from the plates of the bones of the normal and the rachitic rats. These are shown in Figures 10 and 11. It is evident that there is no improvement in structural detail in the lighter prints and that the relative superiority of the infrared plate as regards epiphyseal resolution remains.

## DISCUSSION

The most interesting result shown in the microphotographs is the greater detail evident in the infrared prints than in the ultra-violet and the normal light prints. There is not only a greater wealth of detail shown, but a better and sharper definition of all detail, as stated in the description of the experimental results.

In addition to the presentation of new material, a further discussion may perhaps be permitted. First, it was felt that the prints obtained based on the selective absorption of the bone elements would make it possible to study at leisure the finer structures of the various parts. Such a set of prints would not only differentiate morphological structures but they might also give further information in regard to the color and wave band absorption of the formed elements comprising the test material.

The present observations of the better resolution shown by the infrared light might perhaps be surprising if it were considered only that resolution increases with the numerical aperture of the lens, and also with the shortness of the light wave used.<sup>1</sup> The statement<sup>2</sup> (illustrated with microphotographs) is made in Photomicrography that "unstained whalebone sections appear as a black mass lacking in detail," when photographed with a blue filter, and further, that "the proper procedure is to photograph the object by the light which it transmits. The whalebone sections, for instance, photographed by red light give perfectly satisfactory results, showing ample detail in structure."

Bearing out these views and supplementing the evidence of the usefulness of the infrared studies, Clark<sup>3</sup> wrote: "Some rather opaque specimens show details of internal structure when photographed by the infrared which are not visible in ordinary photomicrographs." From a somewhat different point of view Rawling<sup>4</sup> stated: "Infrared photography has added considerably to the possibilities of microscopy. The advance is due almost entirely to the high transparency to the infrared radiation of many substances which are opaque to visible light. Thus, detail quite invisible to the eye may be disclosed by the infrared plate simply because infrared radiation penetrates the object so easily."

A statement of infrared radiation studies in the physiological field would not be complete without reference to the extensive

investigations of Massopust<sup>5,6</sup> in which various anatomical structures are presented in detail.

Further study of bone structure under varying conditions by the method of microphotography by infrared radiation are in progress.

### SUMMARY

A comparative microphotographic study of unstained sections of normal, rachitic, and partially healed (2 plus) rachitic bones of rats was made by the use of normal, ultraviolet and infrared radiation. Greater wealth of detail and better and sharper definition of all detail in the epiphyseal layers was obtained in the microphotographs made by infrared radiation. The technic used is described in detail.

Thanks are due Dr. Clay R. Murray for his advice and encouragement, especially in the interpretation of the data.

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## DESCRIPTION OF PLATES

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### PLATE 49

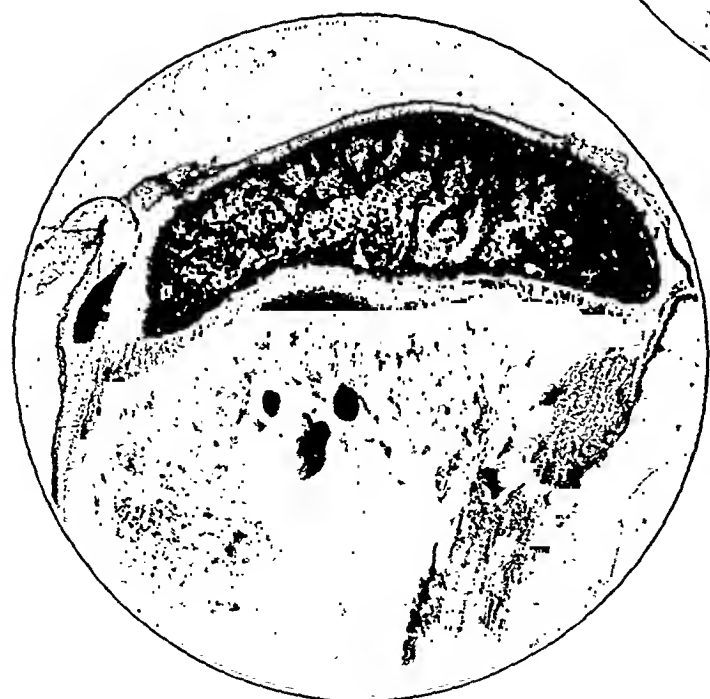
- FIG. 1. Section of femur of normal rat photographed by ultraviolet light (3000-4000 Ångstroms).  $\times$  18.
- FIG. 2. Same section photographed by normal light (4800-6000 Ångstroms).  $\times$  18.
- FIG. 3. Same section photographed by infrared light (7300-9300 Ångstroms).  $\times$  18.



1



2



3

Siegel, Allen, McGuire and Falk

Normal and Rachitic Bone Structure

PLATE 50

FIG. 4. Section of femur of partially healed rachitic rat. Ultraviolet light (3000-4000 Ångstroms).  $\times 18$ .

FIG. 5. Same section photographed by normal light (4800-6000 Ångstroms).  $\times 18$ .

FIG. 6. Same section photographed by infrared light (7300-9300 Ångstroms).  $\times 18$ .







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PLATE 51

FIG. 7. Section of femur from rachitic rat photographed by ultraviolet light (3000-4000 Ångstroms).  $\times 18$ .

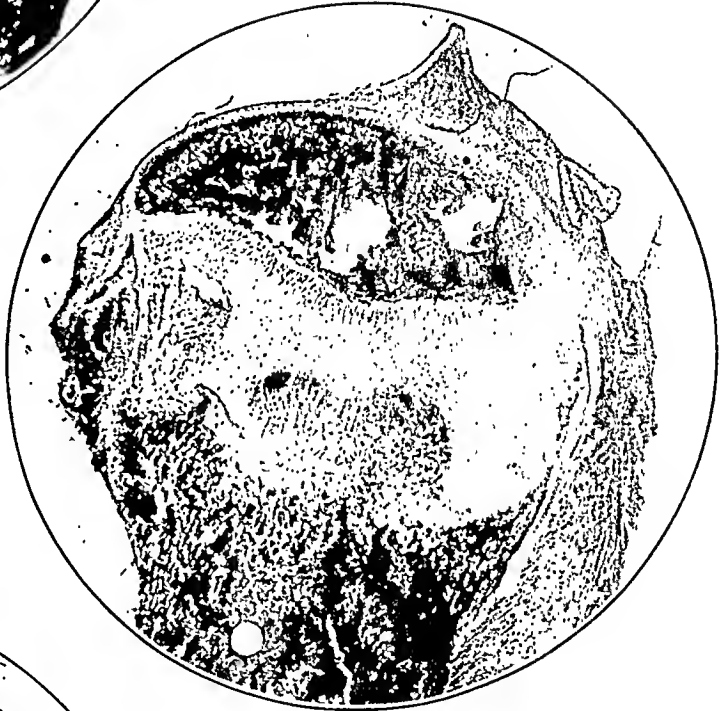
FIG. 8. Same section photographed by normal light (4800-6000 Ångstroms).  $\times 18$ .

FIG. 9. Same section photographed by infrared light (7300-9300 Ångstroms).  $\times 18$ .





7



8



9

Siegel, Allen, McGuire and Falk

Normal and Rachitic Bone Structure

PLATE 52

FIG. 10 a. Section of femur of normal rat photographed by ultraviolet light (3000-4000 Ångstroms). Light print.

FIG. 10 b. Same as Fig. 10 a. Dark print.

FIG. 11 a. Section of femur of rachitic rat photographed by ultraviolet light (3000-4000 Ångstroms). Light print.

FIG. 11 b. Same as Fig. 11 a. Dark print.



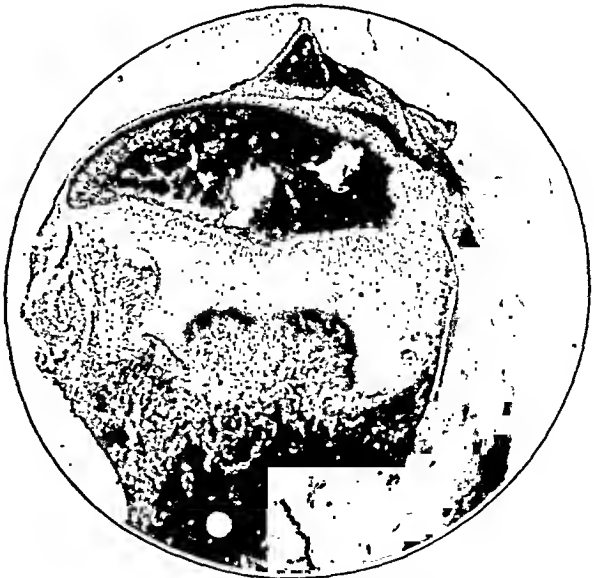
10 a



10 b



11 a



11 b

Normal and Rachitic Bone Structure



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## THE ANTIGENICITY OF THE VIRUS OF TRACHOMA \*

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One of the more important clinical characteristics of trachoma is the tendency of the disease to recur in the same individual. While it is obviously difficult to determine whether repeated attacks of trachoma signify reinfection *de novo*, or instead an exacerbation of the virus remaining quiescent in the tissues during the arrested state, nevertheless the clinical indications are clear that immunity to trachoma must be ineffectual and, if present at all, certainly of low magnitude.

In pursuing studies already reported<sup>1</sup> on the etiology of trachoma the opportunity was presented to make experimental observations in both man and animal on the immunological responses invoked by the virus causing this disease. The observations resulting from this study appear to be of sufficiently broadened interest in the general field of viruses to merit their communication and thus place on record certain information hitherto unavailable.

### METHODS

*The suspension of virus* consisted of tissues scraped from the conjunctiva of clinically active patients during the operation for grattage. The material from the eyes of each patient was suspended in 1.5 cc. of veal infusion broth and later used for testing, either alone or pooled with similar material from other patients.

\* Conducted under a grant from the Commonwealth Fund of New York.  
Received for publication February 2, 1939.

The tissues were ground in a sterile mortar without the use of abrasive, and their infectivity was determined by inoculating monkeys (*Macacus rhesus*) in one eye by swabbing the conjunctiva of the everted lid, and in the other eye by multiple pricking of the conjunctiva with a charged needle and then injecting subconjunctivally 0.2 cc. or more of the suspension. Each test required material fresh from patients since the virus becomes inactivated rapidly.

*Neutralization tests* were performed in general by mixing equal quantities of undiluted or diluted serum and material obtained from patients in the manner described. The mixtures were then incubated in a water bath at 37° C. for 30 to 60 minutes, as will be brought out later, and inoculated in monkeys as indicated above.

*Antiserums* were obtained by injecting intravenously the triturated suspensions of grattage material. Since immunization was dependent on the availability of fresh active tissues rather than on some prearranged schedule, it was fortunate that injections were actually given every 3 to 5 days over a period of 2 months. It was not possible, however, to ascertain the infectivity of the tissues used for immunization because the incubation period of the disease exceeds the period of survivability of the virus.<sup>2</sup> It was felt, nevertheless, that careful selection of patients, prolonged immunization, and the established fact<sup>3</sup> that half the patients taken at random are in the infectious stage, would compensate for this omission. The majority of the tissues injected contained inclusion bodies, so that if, as seems probable, these structures are expressions or phases of the virus itself, they should add correspondingly to the process of immunization. Animals were bled by puncturing the heart 10 days following the final injection, and the serums were subsequently used for the tests to be recorded.

#### EXPERIMENTAL OBSERVATIONS

The determination of possibly acquired immunity in monkeys following recovery from artificially induced infection has already been referred to in a previous communication.<sup>3</sup> In order that the immunological reactions associated with trachoma may be brought together in final and complete form, however, it is desirable to include in this report additional examples bearing on this question. As soon after recovery as active material was available,



monkeys were subjected to inoculation as described above. In order to illustrate the reactions observed, the data from 5 typical animals are given in Table I. It will be seen that in each case infection ensued in somewhat the same manner as observed in the previous instance and ran a similar course. The interval between infection and reinoculation varied from 2 weeks to 3 months, so that no particular emphasis can be placed on the differences in time between recovery and subsequent inoculation as a factor in the development of the later infection. The evidence for continued susceptibility in recovered monkeys can be amplified by numerous examples in this laboratory. In fact, it is because of the frequency of this observation that it is now our custom to reserve

TABLE I  
*Effect of Recovery from Experimental Trachoma on Later Infection*

Animal number	Synopsis, first infection		Interval after recovery	Synopsis, later infection	
	Incubation period	Duration		Incubation period	Duration
	<i>days</i>			<i>days</i>	
4	14	16 months	3 months	10	6 months (died)
2-4	9	6 weeks	2½ months	7	5 weeks
3-5	6	10 weeks	2½ months	18	12 weeks
8	21	11 weeks	2 weeks	6	7 weeks
9	10	10 weeks	3 weeks	17	9 weeks

recovered animals for future experiments, since having been infected successfully in the past they are of proved susceptibility to trachoma and thus automatically exclude in the analysis of data the possibility of individual resistance as responsible for the absence of infection following inoculation of trachomatous tissues.

Subsequent experiments were undertaken to determine, if possible, the occurrence of antibodies in the blood of patients suffering with trachoma. Blood was taken from a number of individuals who had been afflicted with the disease over periods varying from a few months to several years. In some experiments defibrinated blood was used and in others only serum. Mixtures of gramage material and blood, the ultimate concentration of which varied from 1:1 to 1:5, were incubated in a water bath at 37° C. for 30 minutes and then introduced conjunctivally in monkeys. In no instance was it possible to show that the blood of patients neutralized active virus (*i.e.*, prevented infection when inoculated in

this form in susceptible monkeys). The resulting infection in the animals was as typical as that observed in monkeys inoculated with material alone, or material previously mixed and incubated with equivalent amounts of non-trachomatous serums. It is, therefore, fair to state that under the conditions outlined the blood of trachomatous patients contains no antibodies capable of neutralizing the specific virus.

Similar experiments were carried out with the serums of monkeys shortly after their recovery from experimental infection. The results in these instances confirmed the observations made with serums of patients.

It is, however, a well recognized fact among bacteriologists and immunologists interested in ophthalmological infections that infectious agents adapted to the conjunctiva very rarely stimulate formation of antibodies. Functionable antigens, such as those contained in pneumococcus, streptococcus, gonococcus, Koch-Weeks bacillus, and so on, are antigenically inert as far as antibodies in the general circulation are concerned unless, as is rarely the case, the organisms penetrate through the conjunctival barrier. Consequently, lack of measurable protective antibody in trachoma may imply as much an incompetency of the tissues involved to elaborate antibodies as an impotency on the part of the antigen to stimulate them. Thus, then, it became necessary to attempt artificial immunization of animals in order to define more accurately the essential conditions of the general immune response to the virus of trachoma.

Accordingly, 2 rabbits were immunized as described above, and because it may be possible that a virus will stimulate formation of antibodies only in animals susceptible to the infection it induces, monkeys were also studied. Two monkeys that had recovered from severe and prolonged experimental trachoma were used for the purpose. Both rabbits and monkeys received 16 injections of the human tissues as soon after collection from the patient as was possible, each injection consisting of 0.5 cc. or 1 cc. of the suspension. The serums subsequently obtained from these animals were then studied for the presence of neutralizing or protective antibodies.

Neutralization tests were conducted according to the technique described above. The tubes containing the mixtures of tissues

and serums were agitated from time to time during incubation and in each instance it was noticeable grossly that the serums both agglutinated and lysed the human cells. Smears made at the end of the incubation revealed a complete disappearance of red cells and less complete but still marked lysis of polymorphonuclear cells, with lymphocytes somewhat more resistant than the neutrophils. Perhaps as many as half of the epithelial cells from the conjunctiva were also lysed, while the others were in more or less normal condition. Since corollary experiments conducted in a similar manner with normal rabbit and monkey serums revealed no agglutinative or lytic effect on the human cells, this observation suggests at least that the conditions of the experiment were appropriate for the formation of antibodies in response to the active antigens present.

TABLE II

*Protective Capacity of Rabbit and Monkey Antiserums in Experimental Trachoma*

Experiment number	Antiserum		Period of fixation	Result of test
	Animal	Dilution		
NT-3	Rabbit	1:1	min.	No protection
	Monkey	1:1	30 30	No protection
NT-6	Rabbit	1:5	30	No protection
	Monkey	1:3	30	No protection
NT-7	Rabbit	1:1	60	No protection
	Monkey	1:1	60	No protection

Seven experiments were done in all, but in only three was the original material infectious for monkeys. The results of these three experiments are summarized in Table II. It will be seen that rabbit and monkey antiserums were used in parallel tests with the same human tissues. Serums were diluted 1:1, 1:3, and 1:5, and incubations were carried out for 30 and 60 minutes. Immediately on removal from the water-bath the mixtures were inoculated in monkeys, inducing infections typical in every way. Later, experiments were done in which guinea pig complement was added to the system, but also without influencing the infectivity of the virus in any measurable manner. It seems definite, therefore, that prolonged artificial immunization of rabbits or monkeys, the latter of verified susceptibility to trachoma, with human

tissues containing specific virus fails to stimulate antibodies capable of protecting monkeys against experimental trachomatous infection.

### DISCUSSION

The observations recorded in the present communication suggest that judged by increased resistance to experimental infection in monkeys the virus of trachoma is an ineffectual antigen. The lack of active immunity observed so commonly in patients has thus been duplicated experimentally in animals. In fact, because normal monkeys are frequently resistant to trachomatous infection, it has been found an accommodation to use over and over again recovered animals as of proved susceptibility. Records of individual monkeys, consequently, show that in this laboratory animals have been infected successively three, four, and even five times.

Since the serums from both patients and monkeys with trachoma contained no protective antibodies, experiments were undertaken to stimulate a general rather than a localized immunogenic reaction by injecting intravenously tissues from patients containing both active virus and inclusion bodies. While rabbits are resistant to infection and perhaps in consequence unsuited for immunization with the virus of trachoma, the monkeys studied, on the other hand, were of verified susceptibility, having recovered only recently from the experimental ocular infection. Yet their serums, just as those of the rabbits, were lacking in protective antibodies as determined by methods usually adopted for their detection. In the face of such evidence, therefore, it is difficult to escape the conclusion that the virus of trachoma is at best a poor antigen.

The data, however, cannot be interpreted to mean that the virus of trachoma is not an antigen. It is difficult to conceive of any active infectious agent as devoid of antigenicity; it is more probable that the antigen is of extremely low activity and the methods in use at the present time for the demonstration of antibodies are too crude to detect their low concentration. In any case, it must be admitted that whatever immune response is aroused by the virus of trachoma, it is not capable of combatting the active infection arising spontaneously in man or induced artificially in monkeys.

On considering the question of immunity from its obverse aspect, the host, it is obvious that the conjunctival and corneal epithelium (the only tissues involved in uncomplicated trachoma) participate only to a minor degree, if at all, in the elaboration of antibodies. Consequently, unless the infectious agent penetrates through the barrier set up by the conjunctiva, the conditions for active immunity and formation of antibody are only rarely established. In this particular instance the low virulence of the trachomatous virus creates a relatively mild reaction, thus precluding its invasion into the subconjunctival tissues and beyond; even when introduced repeatedly into the general circulation, moreover, it exerts antigenically an inadequate stimulation.

#### SUMMARY AND CONCLUSION

1. Clinical observation reveals very little if any immunity to trachoma.
2. It has not been possible to demonstrate increased resistance to experimental trachoma in monkeys following recovery from the infection.
3. The serum or defibrinated blood of patients with active infections of varying duration exerts no neutralizing or protective effect on the virus of trachoma.
4. The serum of infected or recovered monkeys contains no antibodies demonstrable by the usual methods of protection.
5. The serum of rabbits or susceptible monkeys receiving repeated intravenous injections of active trachomatous tissues contains similarly no antiviral substances.
6. It is concluded that the virus of trachoma is an impotent and ineffectual antigen.

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# THE REACTION TO KILLED TUBERCLE BACILLI IN NORMAL AND IMMUNIZED (SENSITIZED) RABBITS \*

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The purpose of these experiments was to determine how the reaction to intraperitoneally injected killed tubercle bacilli is modified by previous skin injections of the same material. When living bacilli are injected into an experimental animal two parallel processes develop, namely, the growth of the injected organisms and the progressively changing response of the host. With the use of killed bacilli the first factor is eliminated. This simplification of the problem is especially desirable when the attempt is made to study the regressive phase of the cellular reaction. Approximately half of our rabbits received repeated intracutaneous or subcutaneous injections of killed tubercle bacilli before intraperitoneal injections were made, and the resulting sensitization was measured by tuberculin tests or by the reaction to killed tubercle bacilli or by both. The others received only intraperitoneal injections and served as controls.

## EXPERIMENTAL PROCEDURE

Bovine tubercle bacilli of the virulent Ravenel strain grown on glycerin agar by Dr. Jules Freund were carefully removed from the surface of the culture and killed by heat at or above 100° C. The death of the bacilli was confirmed by injecting 5 mg. in suspension subcutaneously into guinea pigs. We used unbuffered normal saline as the diluent and the pH of the resulting suspensions was between 6.8 and 7.2. The suspensions were homogeneously opaque on gross examination, but small clumps of bacilli were found under the oil immersion lens.

Tuberculin tests were made by intracutaneous injection of 0.04 cc. of old tuberculin (human type) diluted to 0.2 cc. with 4 parts of saline.

The skin reactions after the intracutaneous injection of killed

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bacilli or of tuberculin were examined in 48 hours and designated as negative when the largest diameter of edema was less than 5 mm.; 1 plus, when it was from 5 to 9 mm.; 2 plus, from 10 to 19 mm.; 3 plus, from 20 to 29 mm.; 4 plus, from 30 to 39 mm.; and 5 plus, from 40 to 49 mm., in accordance with the procedure used in this laboratory.

Of 83 rabbits used in the experiments, 17 were eliminated by "snuffles," pneumonia or diarrhea before intraperitoneal injection was performed. Two others died after peritoneal injection, 1 from pneumonia and the other from diarrhea. Two animals with extensive subcutaneous caseation but no peritoneal lesion were not included because it was evident that the inoculating needle had not entered the peritoneal cavity. The remaining 62 rabbits form the basis of this report. In most of the animals 5 cc. of saline was injected into the peritoneal cavity and the syringe containing the killed bacilli was then attached to the same needle. One cubic centimeter of a suspension containing 10 mg. of killed bacilli was injected and the lumen of the needle was rinsed into the peritoneum with 2 cc. of saline. In 3 animals (Nos. 9, 10 and 16) the suspension alone was injected. During the course of the experiment all of the animals except No. 31 increased in weight.

#### DESIGNATION OF EXTENT OF PERITONEAL LESIONS FOUND AT AUTOPSY

The lesions found at autopsy were rated largely by the number and size of the tubercles in the peritoneum and omentum, but in rabbits that lived a week or less the redness of the omentum and the amount of intraperitoneal fluid were considered. The extent of tuberculosis is indicated as follows:

Tuberculosis was recorded as absent when no tubercles were found on gross or microscopic examination.

The lesion was recorded as doubtful when microscopic sections revealed groups of mononuclear cells, although gross examination revealed no tubercles or a very few indistinct fibrotic nodules on the peritoneum, with no change in the omentum.

One plus was used to indicate the presence of a few clearly defined tubercles with histological characteristics of tuberculosis in the peritoneum or the omentum and perhaps elsewhere.

Two plus was used when there were tubercles in moderate num-



ber, perhaps 10 small or 5 larger ones, on the peritoneum and omentum. The omentum in some instances contained a considerable amount of uninvolved tissue. In these animals and in those with more extensive lesions it was usual to find a caseous mass at the point where the needle perforated the peritoneum.

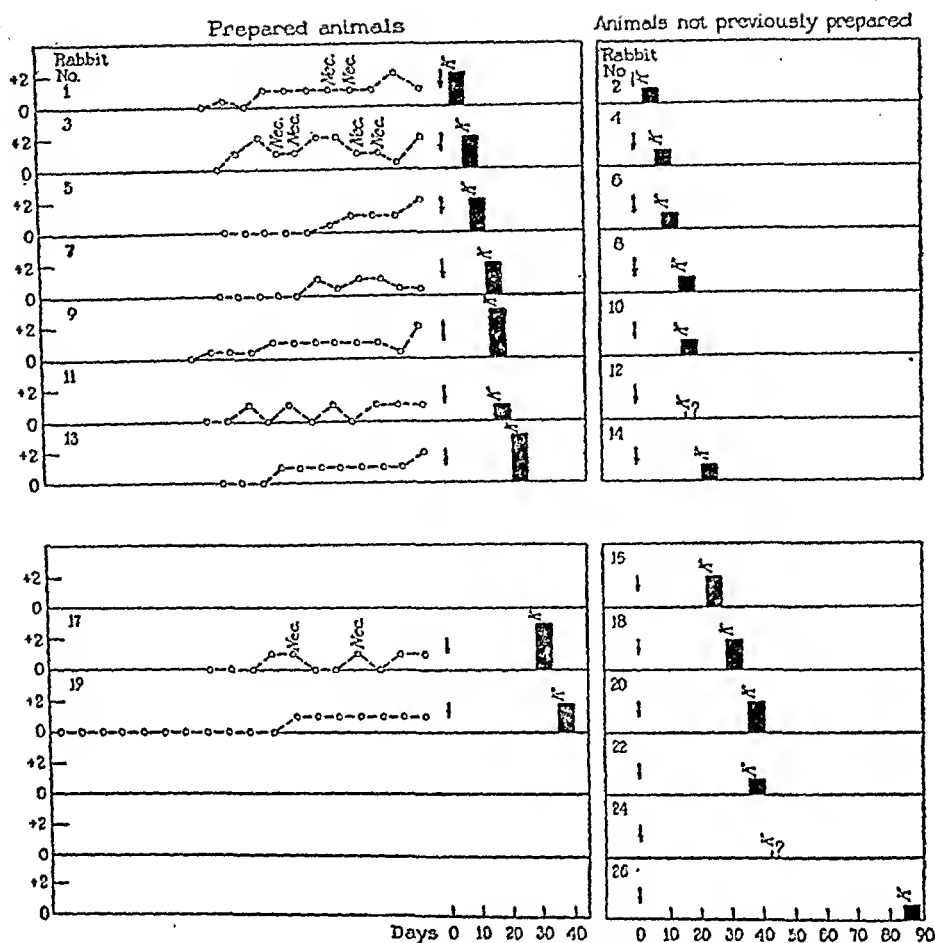
Three plus was used when there were many tubercles on the parietal and visceral peritoneum and the omentum, the latter being usually much thickened and uniformly matted. The number of tubercles under the dome of the diaphragm gave a measure of the extent of peritoneal involvement.

Four plus was used when the parietal peritoneum and the peritoneal surfaces of the liver, spleen, intestines and at least the greater part of the omentum were studded with tubercles. The peritoneal surface of the diaphragm was studded with discrete tubercles or was covered by a confluent mass of tuberculous tissue.

### EXPERIMENT I

Tubercle bacilli were autoclaved for 15 minutes at 15 pounds pressure, dried over phosphorus pentoxide and suspended in saline so that 2 mg. of dry bacilli (which were estimated to represent 10 mg. of moist bacilli) were present in each cubic centimeter. They were then placed in an Arnold steam sterilizer for 30 minutes at 98° C. All but 2 of the treated animals were given 10 to 12 intracutaneous injections each of 0.5 mg. of killed bacilli at approximately weekly intervals. Rabbit No. 19 received 18 injections of the same quantity and rabbit No. 9 received 6 injections of 0.2 mg., 5 of 0.5 mg., and 1 of 1 mg. The graphs in Text-Fig. 1 which give the extent of edema produced by these injections show the progress of sensitization. No tuberculin was used.

Sensitization in all of the animals of this experiment was scant and the diameter of the swelling produced by the intracutaneous injection of heat killed tubercle bacilli in no instance exceeded 10 to 19 mm. Nevertheless the extent of peritoneal tuberculosis produced by the injection of a large quantity of heat killed tubercle bacilli was uniformly greater in sensitized than in untreated animals. In this experiment sensitization was measured only with heat killed tubercle bacilli and in the absence of tuberculin tests it is not possible to determine the effect of the intra-peritoneal injection or sensitization.



TEXT-FIG. 1. Lesions present in prepared rabbits of Experiment 1 and in rabbits not previously treated with killed tubercle bacilli. The broken lines show the extent of the edema produced by the intracutaneous injection of killed bacilli. "Nec." indicates that caseous necrosis was found at the site of intracutaneous injection in 7 days or less. The arrow indicates the day on which the intraperitoneal injection of 10 mg. of killed tubercle bacilli was made. The position of the black column indicates the duration of life, while its height is proportional to the extent of lesions at autopsy. "K" indicates the day animal was killed.

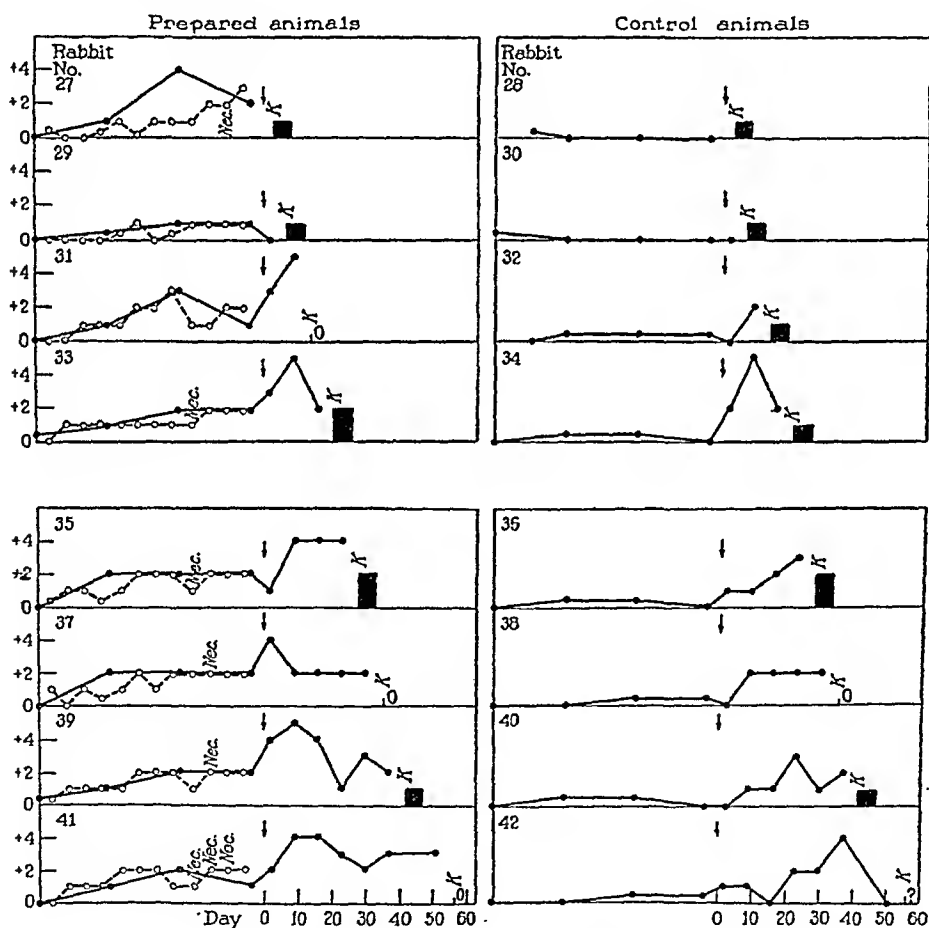
## EXPERIMENT 2

In Experiment 2 the suspension of killed bacilli was made by weighing living tubercle bacilli, grinding them and adding saline so that 10 cc. contained 1 mg. of bacilli. It was sterilized in a loosely stoppered bottle in an Arnold steam sterilizer for an hour at 100° C. It was homogeneous on gross examination. Of 12 intracutaneous injections of killed bacilli the first was 0.4 mg., and each of the 11 subsequent injections was 0.2 mg., given at inter-

vals of 5 or 6 days. The reactions to the intracutaneous injections of killed bacilli and to tuberculin are shown in Text-Fig. 2. Eight pairs of white rabbits were used for this experiment, all animals being over 2 kilos in weight.

In this experiment tuberculin tests were made at intervals of approximately 2 weeks. The increase of sensitization following intraperitoneal injection is evident in almost all experiments and in general is more prompt and reaches a higher level in sensitized than in control animals.

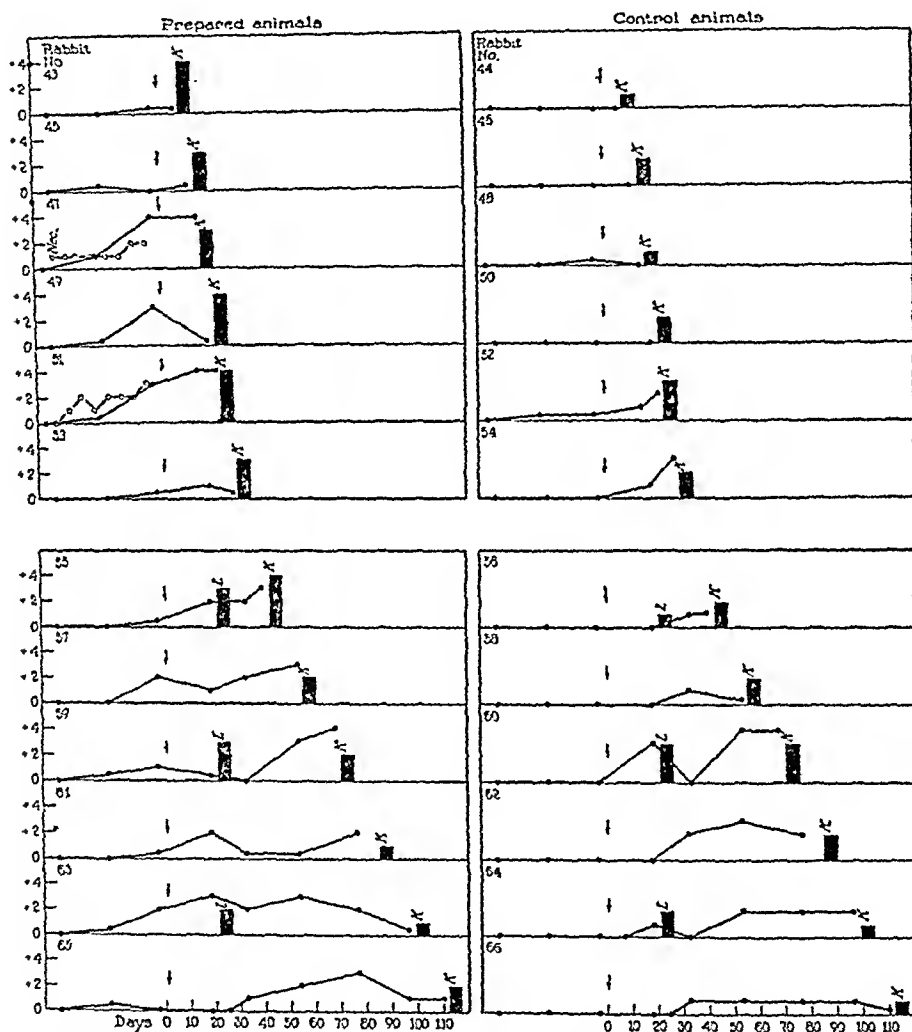
In this experiment the extent of lesions following peritoneal injection is not conspicuously different in prepared and in the control animals.



TEXT-FIG. 2. Lesions of prepared and control rabbits of Experiment 2. The broken lines represent extent of reactions to intracutaneous injections of killed bacilli, the solid lines that of reactions to old tuberculin. The arrow indicates the day on which intraperitoneal injection of killed bacilli was made. The black columns indicate the length of life and the extent of lesions at autopsy.

## EXPERIMENT 3

The heat killed tubercle bacilli were prepared as in Experiment 2. The sensitizing injections were given in two different ways. Two Havana rabbits (Nos. 47 and 51) were given 2 simultaneous intracutaneous injections of 0.2 mg. of killed bacilli fol-



TEXT-FIG. 3. Lesions of prepared and control rabbits of Experiment 3. The graphs are drawn as in Text-Fig. 2. The black columns indicate the extent of lesions at autopsy (K), and the extent of lesions observed at laparotomy (L) in 6 animals.

lowed after 5 days by another double injection and 6 single injections of the same quantity at intervals of 5 or 6 days. The other 10 prepared rabbits, which were of gray stock, were given subcutaneously 10 injections each of 0.4 mg. of killed bacilli at

the same intervals. In this experiment a suspension of 10 mg. of killed tubercle bacilli was injected through a blunt pointed needle inserted through the abdominal wall in the left lower quadrant.

Three treated rabbits (Nos. 55, 59 and 63) and their controls (Nos. 56, 60 and 64) were subjected to exploratory laparotomy 21 days after the intraperitoneal injection of killed tubercle bacilli (see Text-Fig. 3). In 1 pair the peritoneal lesion of the sensitized animal (No. 55) was more extensive than that of the control (No. 56), but in both of these animals lesions were more extensive 21 days later when autopsies were performed. In the other 2 pairs sensitized and control animals had equally extensive lesions at the time of operation, but with a longer period between operation and death lesions were less extensive at autopsy than at operation.

In this experiment (Text-Fig. 3) there was, just before intraperitoneal injection, some but not a conspicuous difference in sensitization between the animals prepared by injections of heat killed tubercle bacilli and the controls, but following this injection sensitization in general increased more rapidly and reached a higher level in prepared than in control animals. In this experiment peritoneal lesions were much more extensive in prepared than in control animals.

#### LESIONS PRODUCED BY INTRAPERITONEAL INJECTIONS OF HEAT KILLED TUBERCLE BACILLI

*Lesions of the Omentum:* Dead tubercle bacilli injected into the peritoneal cavity produce more thickening of the omentum in animals that have been given preliminary skin inoculations than in their controls. The omentums of 8 prepared animals (Nos. 3, 5, 7, 13, 17, 33, 47 and 65) were thick, while the omentums of their controls were thin. In only 1 pair was the omentum of the control (No. 32) thick when the omentum of the corresponding prepared animal (No. 31) was not thickened. Prepared animal No. 19 with a thin omentum had 2 controls — 1 with thickened omentum (No. 20) and 1 with a thin omentum (No. 22). In the remaining animals the omentums of prepared animals were approximately the same as those of the controls.

Tubercles were present in large numbers in 13 (45 per cent) of the immunized and in only 2 (7 per cent) of the control animals. Tubercles were present in moderate numbers in 5 (17 per

cent) of the immunized animals and in 7 (23 per cent) of the controls. Tubercles were present in small numbers in 3 (10 per cent) of the immunized animals and in 10 (33 per cent) controls. No tubercles were present in the omentums of 8 immunized and 11 control rabbits.

The omentum of each of the 29 prepared animals and of the 30 controls was examined microscopically. After 3 weeks the control animals are sensitized by the peritoneal injection and hence the animals have been divided into two groups: those that lived 21 days or less and those that lived longer. In the 15 pairs of animals living from 3 to 21 days there were conspicuous differences between the previously treated and the control animals, whereas among the animals surviving from 23 to 112 days controls and treated animals differed little.

Of the rabbits living 3 weeks or less the omentums of 3 prepared animals (Nos. 11, 27 and 31) and of 6 controls (Nos. 6, 8, 12, 28, 34 and 48) showed insignificant cellular reactions. The omentums of 2 of the prepared animals (Nos. 1 and 29) contained loose groups of small undifferentiated monocytes. A scant cellular reaction with accumulation largely of polymorphonuclears, in part necrotic, surrounded by monocytes and in some instances by fibroblasts, occurred in 4 control animals (Nos. 2, 4, 30 and 44). Fully formed tubercles were found in the omentums of 6 prepared animals (Nos. 3, 5, 7, 9, 43 and 45) and in 3 controls (Nos. 10, 32 and 46). By fully formed tubercles is meant compact masses of cells that have assumed the characteristics of epithelioid cells with cell body and nucleus both larger than those of the earlier monocytes. These lesions have the microscopic characteristics of tubercles as found in experimental animals and in man. Necrotic foci are usually present, the necrotic material in some cases containing abundant nuclear fragments, in others containing no remains of nuclei. Acid-fast bacilli are frequently found. It is significant that either loose groups of undifferentiated monocytes or well formed tubercles were present in the omentums of 12 of the 15 prepared rabbits living from 3 to 21 days, but were present in only 5 of the 15 controls living for the same period.

There were 14 prepared animals and 15 controls living from 23 to 112 days and among these animals there was scant difference between prepared and control animals. The omentums of 4 pre-

pared animals (Nos. 37, 41, 61 and 63) and 4 controls (Nos. 22, 38, 42 and 64) showed an insignificant cellular reaction. The omentum of 1 prepared animal (No. 51) and its control (No. 52) contained fully formed tubercles with necrotic foci. In the omentums of 9 prepared rabbits and 10 controls tubercles were regarded as regressive because there was formation of fibrous tissue within them; epithelioid cells had increased in size and central clear areas had appeared in the cytoplasm near the nuclei. Between these large cells with irregular outline were fine fibrils. In the omentums of the animals that lived longest epithelioid cells had decreased in number and giant cells were numerous. A progressive increase in fibrous tissue occurred, sometimes with increased vascularity. Necrosis and acid-fast bacilli were present in the omentums of 9 of the 14 prepared animals of this group. Necrosis and acid-fast bacilli were present in the omentums of 6 of the 15 control animals of this group.

The cellular reaction is greater and the formation of characteristic lesions in the omentum occurs earlier in immunized than in control rabbits. Even more conspicuous is the larger size of the lesions in the immunized and sensitized rabbits. An increased cellular reaction in sensitized animals inoculated with living tubercle bacilli has been repeatedly observed. Nichols<sup>1</sup> injected tubercle bacilli intravenously and found that tubercles developed earlier in the lungs of rabbits that had been previously immunized with avirulent bacilli than in the unprepared controls. Gardner<sup>2</sup> injected guinea pigs intraperitoneally with living tubercle bacilli and found that tubercle formation in immunized animals after 4 to 6 days was in the same stage as in controls after 10 days.

*Lesions of Parietal and Visceral Peritoneum:* Necrosis evident on gross examination was present at the point of peritoneal injection in 62 per cent of the immunized animals and in 43 per cent of the controls. This necrosis was found especially with the more advanced lesions.

A large number of tubercles was present on the peritoneum of 12 (41 per cent) of the immunized animals but in only 1 (3 per cent) of the controls. Tubercles were present in moderate numbers in 5 (17 per cent) of the immunized animals and in 10 (33 per cent) of the controls. Tubercles in small numbers were found in 7 (24 per cent) of the immunized animals and in 17 (57 per

cent) of the controls. In 5 immunized and 2 control animals of the first 2 experiments, no peritoneal tubercles were present.

Prudden and Hodenpyl in 1891<sup>3</sup> injected into the peritoneal cavities of rabbits 2 to 3 cc. of a milky suspension of tubercle bacilli killed by the heat of the autoclave and found creamy masses surrounded by epithelial cells and fibrous tissue, but stated that caseation did not occur. Branch and Cuff<sup>4</sup> reported death of guinea pigs following repeated injections of large quantities of killed bacilli into the peritoneum.

Killed tubercle bacilli have been intravenously injected into rabbits by many observers. Prudden and Hodenpyl<sup>3</sup> found tubercle-like masses in the lungs, liver and spleen but none in the kidneys. Masur<sup>5</sup> reported the formation of giant cells without caseation in the lungs of rabbits. Kelber,<sup>6</sup> supported by Baumgarten, maintained that killed bacilli caused a reaction like that produced by a foreign body and no true tubercle formation. Krompecher<sup>7</sup> injected from 1 to 8 cc. of a suspension of killed bacilli and found caseation when the bacilli used were of a virulent strain. Coulaud<sup>8</sup> found tubercles in the lungs of animals injected with suspensions of killed bacilli which were not homogeneous, but found none if the suspensions were homogeneous.

*Lesions Outside of Peritoneum:* Masses of epithelioid cells occupying wide areas of the tissue were found in about half of the retrosternal nodes that were sectioned and were found both in prepared and in control animals of Experiment 1. Acid-fast bacilli were found only once and doubtless reached these nodes by way of lymphatics.

Groups of epithelioid cells were found in the spleen and liver in more than half of the animals of this experiment, about equally in prepared and in control animals. Groups of similar cells were occasionally found in the lungs but they were not found in the kidneys. These nodules, composed of cells resembling those of tubercles, are evidently caused by dead tubercle bacilli disseminated by the blood stream.

*Relation of Sensitization to the Extent of Lesions:* It is evident that the procedure used in Experiment 1 gives no adequate insight into the progress of sensitization in animals that have received repeated cutaneous injections of heat killed tubercle bacilli preceding the massive intraperitoneal injection, and has given no



information about sensitization following this injection. In Experiments 2 and 3 sensitization was measured by tuberculin tests repeated at intervals throughout the course of the experiment. It is noteworthy that intraperitoneal injection of heat killed tubercle bacilli is almost invariably followed after a week or more by much increased sensitization, and sensitization reaches a higher level in previously treated animals than that found in control animals that have had no preparation preceding the intraperitoneal injection. Nevertheless it is evident that the control animals as well as the animals prepared by intracutaneous or subcutaneous inoculation are ultimately almost equally sensitized, for after the 3rd week following intraperitoneal injection the difference between the two is insignificant.

In comparing the lesions found in sensitized and in unsensitized animals it should be noted that the two differ conspicuously only during the period shortly after intraperitoneal inoculation. Hence a sharp difference between the two cannot be expected.

### CONCLUSIONS

When heat killed tubercle bacilli are injected into the peritoneal cavity of rabbits, formation of tubercles and of tuberculous tissue occurs more quickly and becomes more advanced in animals that have received preliminary intracutaneous or subcutaneous injections of killed tubercle bacilli than in their unprepared controls. The difference is greatest 3 or 4 weeks after intraperitoneal injection when the lesions in prepared and in control animals are at their height. After 5 weeks no difference in the two groups is evident and later the lesions in both regress.

The extent of tuberculous lesions is greater in the prepared animals which are both immunized and sensitized than in their controls, but there is no close correlation between sensitization measured by the tuberculin reaction and the extent of tuberculous lesions.

Heat killed tubercle bacilli cause necrosis with the characteristics of caseation both in control (unprepared) animals and in those that have received preparatory injections, but it is more frequently found in the latter. Caseation is in general more advanced in sensitized animals but there is no exact correlation between sensitization and caseation.

When killed tubercle bacilli are injected into the peritoneal cavity, tubercle-like nodules composed of epithelioid cells are often found in the retrosternal lymph nodes, spleen, liver and lungs of both immunized and control animals.

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## SKIN TUMORS FOLLOWING A SINGLE APPLICATION OF METHYLCHOLANTHRENE IN C57 BROWN MICE\*

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Connective tissue tumors have been produced with extremely small amounts of carcinogenic hydrocarbons.<sup>1</sup> A few tumors have followed the application of a single drop of hot tar to mouse skin.<sup>2</sup> It is generally believed that protracted painting with carcinogenic agents is necessary for the development of skin neoplasms. Fieser states that "in order to produce skin tumors in mice with even a highly potent carcinogen in benzene solution some 30 deliberate applications must be made."<sup>3</sup> This statement may be true for many strains of mice. Kreyberg<sup>4</sup> and Bonser<sup>5</sup> have established strains by selective breeding whose skin is especially sensitive to the action of carcinogenic substances.

In investigating the influence of methylcholanthrene on the development of internal tumors in mice the chemical was applied successively at 9 different sites.<sup>6</sup> Papillomas were found at the site of the first painting within 4 to 5 weeks among mice of the C57 brown strain. Investigation of this phenomenon seemed warranted.

C57 brown mice were obtained from the Roscoe B. Jackson Memorial Laboratory where the strain originated in 1925. They have a relatively high incidence of spontaneous internal tumors and less than 5 per cent of mammary carcinoma. The mice were painted when 4 to 5 weeks old with methylcholanthrene 0.5 per cent in benzene. It was applied to the back, from the occiput to the lumbar region, with 2 strokes of a No. 8 camel's hair brush. The animals were not allowed to breed. Sex distribution was equal. They were kept in glass cages and were fed Purina dog chow, water being available at all times.

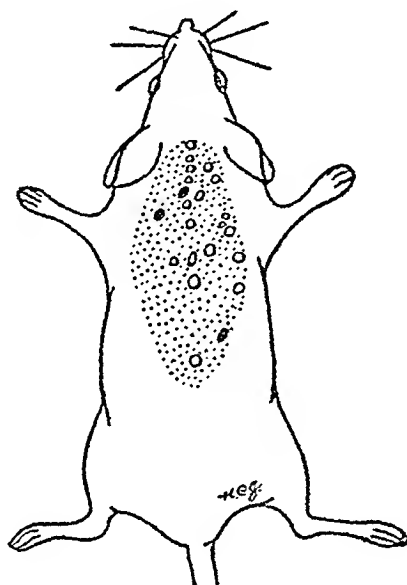
Forty-four mice were painted with methylcholanthrene. Epilation occurred within 10 days. Ulceration was rarely found. Six-

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teen of the animals developed a total of 22 papillomas in the painted area (Text-Fig. 1). The first tumor appeared in each mouse between the 31st and 48th days after painting. These lesions had all the gross characteristics of benign skin tumors produced by multiple paintings with carcinogenic hydrocarbons (Fig. 1). They varied from 2 to 8 mm. in diameter, grew rapidly at first and then passed into a stationary period during which their appearance was not altered. Marked variation in the duration of



TEXT-FIG. 1. Distribution of skin tumors in the area painted once with methylcholanthrene 0.5 per cent in benzene. Stippling indicates the painted area; circles represent papillomas; dots indicate carcinomas.

the tumors was noted (Table I). Fifteen of the papillomas regressed completely. The sites of the tumors showed no gross evidence of scarring after the lesions had disappeared. Hair eventually grew in the epilated areas. It was frequently white instead of brown.

Of the 7 papillomas that remained, 3 became malignant and 4 persisted beyond the 225th day after painting. The latter did not change in appearance for more than 100 days, and showed no evidence of progressive growth. The 3 carcinomas occurred in 1 male and 2 female mice on the 228th, 114th, and 124th days respectively (Fig. 2). Diagnosis was confirmed histologically, invasion of muscle being used as the criterion of malignancy (Fig. 3). No metastases were noted but the mice were sacrificed

as soon as clinical evidence of carcinoma was present. In contrast with mice painted twice weekly with methylcholanthrene 0.5 per cent in benzene, these animals remained in good health throughout the experiment.

This reaction of C57 brown mice is probably another instance of genetically determined increase in tissue susceptibility to an ad-

TABLE I  
*Skin Tumors Following a Single Application of Methylcholanthrene in C57 Brown Mice*

Number of mouse	Sex	Papillomas		Duration
		Appeared	Disappeared	
		<i>days</i>	<i>days</i>	<i>days</i>
1	M	34	102	68
2	M	46	81	35
3	M	38	94	56
4	M	48	76	28
5	M	48	76	28
6	M	41	107	66
		60	105	45
7	F	46	56	10
8	F	39	145	106
9	F	34	76	42
10	F	39	165	126
		42	137	95
11	F	32	105	73
		54	108	54
12	M	26	Carcinoma at 228 days	
		50	72	22
13	F	35	Carcinoma at 114 days	
14	F	31	Carcinoma at 121 days	
15	M	42	Persists at 225 days	
16	M	35	Persists at 225 days	
		54	Persists at 225 days	
		60	Persists at 225 days	

verse environmental stimulating factor. It differs from those produced by Kreyberg and Bonser only in the degree of susceptibility. This is in accord with the concept that carcinoma is not handed down in heredity as such. Cellular susceptibility or resistance to environmental factors is probably the mechanism concerned. A second explanation of the phenomenon would entail a change in the growth and differentiation of skin epithelium by long continued contact with methylcholanthrene. This seems less probable. The fluorescence associated with the chemical cannot be detected in the skin by ultraviolet light 1 week after its application. This is a

crude test and better analytical methods may yield a different result. Furthermore, it is entirely conceivable that a metabolite of methylcholanthrene may initiate neoplasia. Additional experimentation is necessary for the solution of this problem.

The production of skin tumors by a single painting with methylcholanthrene in C57 brown mice appears to afford further opportunity for the investigation of the fundamental physiological changes involved in carcinogenesis. Attempts to affect the biology of the benign tumors may be worth while.

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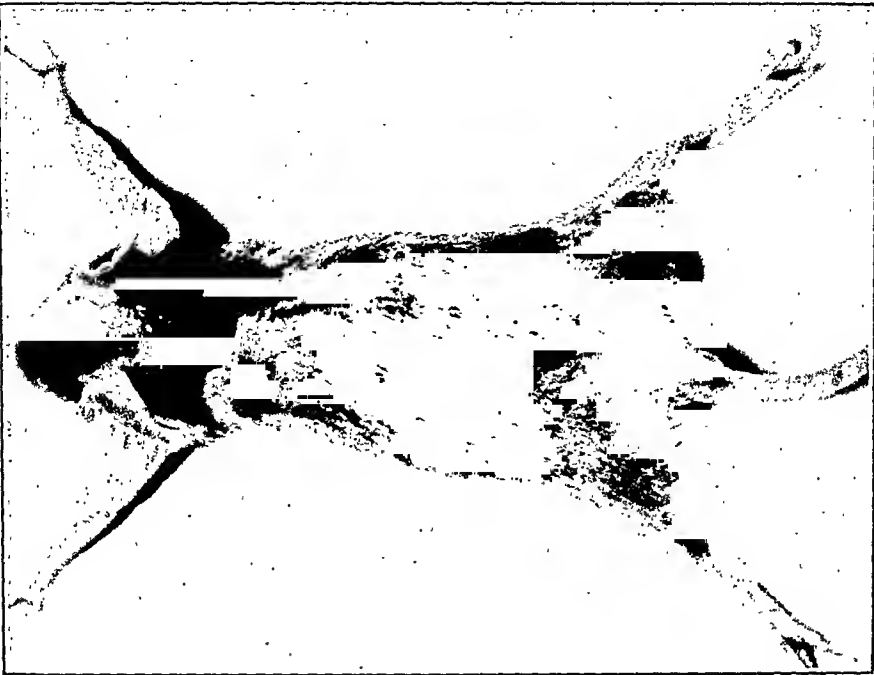
### DESCRIPTION OF PLATES

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#### PLATE 53

FIG. 1. Papillomas in a C57 brown mouse painted once with methylcholanthrene 0.5 per cent in benzene. These tumors have been present for more than 225 days.

FIG. 2. Carcinoma in a C57 brown mouse painted once with methylcholanthrene 0.5 per cent in benzene. A malignant tumor appeared 228 days after painting.



2



1

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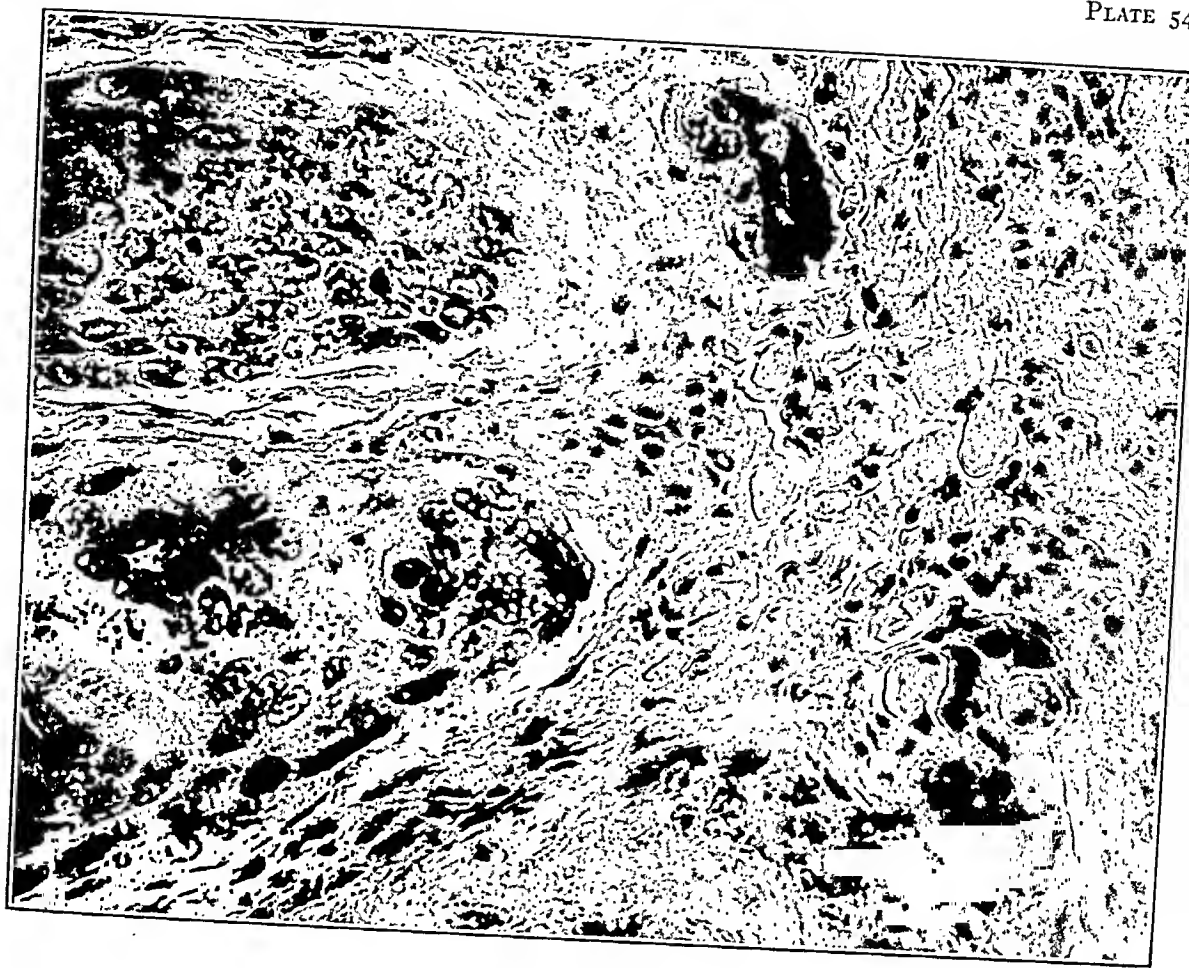
Skin Tumors in C57 Brown Mice

PLATE 54

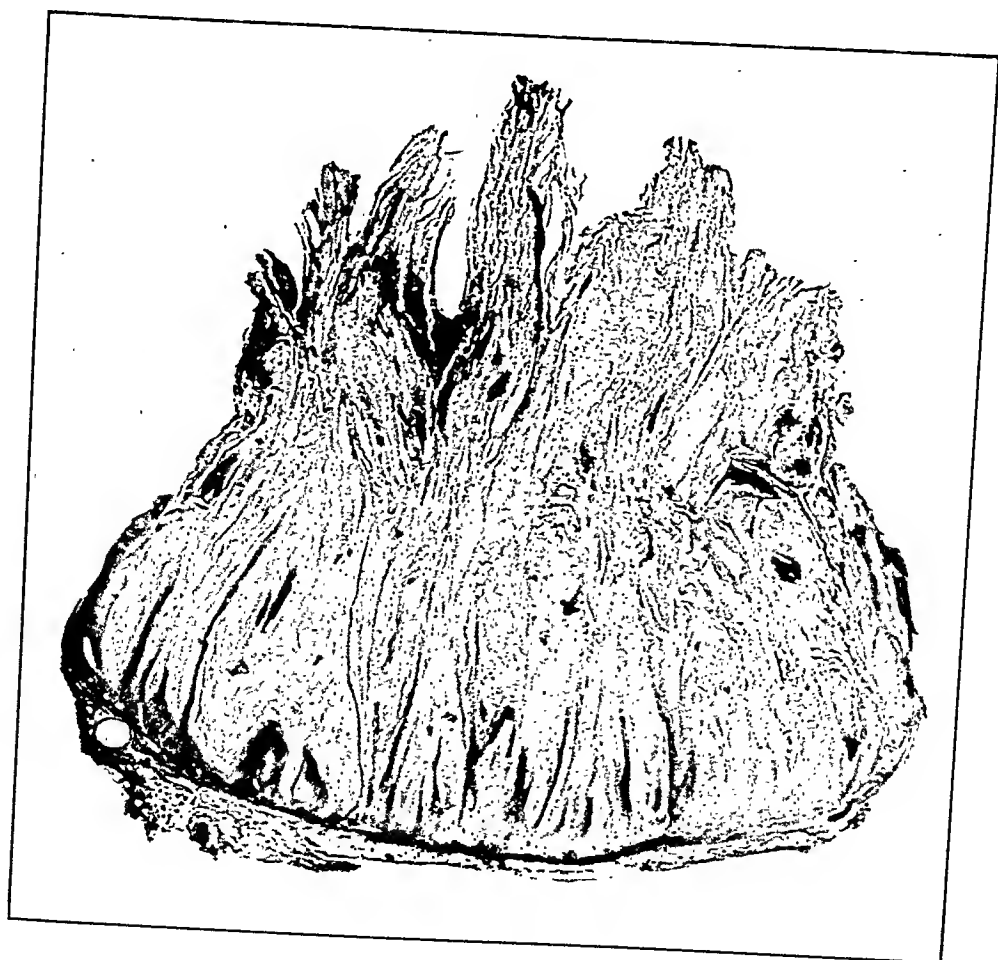
FIG. 3. Microphotograph of carcinomatous growth shown in gross in Fig. 2. The tumor cells have invaded the muscle.  $\times 600$ .

FIG. 4. Microphotograph of a papilloma of the skin arising in a C57 brown mouse after a single application of methylcholanthrene 0.5 per cent in benzene.  $\times 10$ .





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4

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Skin Tumors in C57 Brown Mice



# STUDIES ON THE PATHOGENICITY AND CYTOLOGICAL REACTIONS OF THE SUBMAXILLARY GLAND VIRUS OF THE GUINEA PIG \*

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The submaxillary gland virus of guinea pigs, according to accounts in the literature, is mild in its effects when inoculated into any part of the body with the exception of the brain.<sup>1-6</sup> In this last location it produces fatal results. The virus we planned to use in a series of experiments failed to act in this manner and fatal results followed injections made subcutaneously, intraperitoneally, into various visceral organs, the submaxillary gland, and also into the brain. It might be assumed from this that we had found a highly virulent strain of the virus. However, the animals that were available for inoculation came from a colony maintained by the Genetics Department of Iowa State College and were proved to be completely free of the disease. In view of these circumstances several problems arose. First, was the difference in behavior of the virus due to a greater virulence than that found by other investigators, or to a greater susceptibility of the guinea pig stock employed. Also, were the histological and cytopathological effects of this virus, which was fatal, different from the reactions described for other submaxillary gland viruses of guinea pigs which were milder in their action. A study of these problems forms the first part of this paper. The latter part deals with stages in the development of inclusion bodies and the time required for each phase. The cytological study was supplemented by use of the ultracentrifuge and the results are compared with those of previous experiments with centrifugation.<sup>7, 8</sup>

## MATERIALS AND METHODS

Guinea pigs carrying the submaxillary gland virus were obtained from a dealer in southern Illinois. Uninfected animals

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† We are grateful to the Graduate School of Iowa State College for subscribing to a room at the Marine Biological Laboratory where part of this work was done.

were obtained from the Genetics Department of Iowa State College. Virus for injection was prepared by grinding extirpated sub-maxillary glands in a sterile mortar which contained a small quantity of sterile sand and Locke's solution. Light centrifugation brought down the heavier particles. The supernatant fluid was injected without filtration; its sterility was tested by inoculation on serum agar and on a broth medium.

Tissues removed from animals were fixed in Zenker's fluid plus acetic acid or in a fixative that permitted subsequent testing for thymonucleic acid according to the procedure given by Cowdry.<sup>9</sup> The stains used were Ehrlich's hematoxylin-eosin and Giemsa's method.

The ultracentrifuge was of the type originated by Henriot and Huguenard and developed by Beams and Pickels.<sup>10,11</sup> It was made of duralumin and was operated by air from a tank in which the pressure varied from 95 to 120 pounds per square inch.\* Revolutions per second of the rotor were determined by matching pitch from a Galton's whistle to the loudest audible note from the centrifuge. It had been observed by Beams<sup>10</sup> that the loudest tone from the rotor corresponds in frequency to the rate of rotation. The results indicated speeds of about 2900 to 3900 revolutions per second from which it was calculated that the centrifugal forces were 300,000 to 550,000 times gravity.

Fresh tissues were placed in the rotor and centrifuged at these speeds for from 30 to 60 minutes, and were then removed and fixed immediately. Uncentrifuged control tissues were prepared as previously described<sup>7</sup> to determine the extent of autolysis due to the time factor involved in centrifugation and to compression effects caused by centrifugation.

## I. EXPERIMENTS AND OBSERVATIONS

### *Results from Virus Passages*

*Susceptibility of Different Groups of Guinea Pigs:* An experiment was designed to ascertain if guinea pigs from a stock of animals that carried the virus (Group I, Table I) were less susceptible than those from a stock in which no virus infection had been present (Groups II and III, Table I).

\* We are indebted to Prof. L. T. Brown who kindly permitted us to carry on the centrifugation experiments in the steam and gas laboratories.

TABLE I

*Data on Susceptibility to the Submaxillary Gland Virus as Observed in Three Groups of Guinea Pigs*

Type of stock used	Age	Number of guinea pig	Duration of life after inoculation into submaxillary gland	Pathological lesions in submaxillary gland		
				Histopathology	Intranuclear inclusions	Duct cells
Animals from a colony carrying the virus	Group I 3 weeks	104	days	Slightly congested Congested Slightly hemorrhagic Hemorrhagic Congested Congested	Monocytes	Duct cells
		105	26			
		106	17			
		107	13			
		108	14			
		109	25			
			22			
			av. 19.5			
Animals from a colony not carrying the virus	Group II 3-4 weeks	110	11	Hemorrhagic and autolyzed Hemorrhagic and autolyzed Hemorrhagic and autolyzed Hemorrhagic and autolyzed Hemorrhagic and autolyzed	Numerous Numerous Numerous Numerous Numerous	(105)† (400+)† (90) Unsatisfactory† (108)
		112	14			
		113	13			
		114	9			
		115	8			
			av. 11			
Animals from a colony not carrying the virus	Group III Adults	116	8	Hemorrhagic and autolyzed Hemorrhagic and autolyzed Hemorrhagic and autolyzed Hemorrhagic and autolyzed	Numerous Numerous Numerous Numerous	Unsatisfactory (78) (56) Unsatisfactory
		117	10			
		118	9			
		119	10			
			av. 9.3			

\* Figures in this group represent averages of counts made on two sections.

† Figures inside of parenthesis represent rough approximations after corrections have been made to obtain approximately equivalent size of section, necessitated by extensive tissue destruction.

‡ Unsatisfactory because the severe tissue destruction and autolysis made reliable estimates impossible.

The virus inoculum consisted of 1 part of triturated submaxillary gland and 10 parts of Locke's solution. In preparation for the injection the animals were etherized and an incision was made on the ventral side of the neck to expose the submaxillary glands. A portion of the left gland was removed and fixed. Into the remainder of this gland 0.05 cc. of virus was injected, and into the whole gland on the opposite side 0.1 cc. A total of 0.15 cc. of virus solution was thus injected. All the gland tissues removed before injection proved to be free from intranuclear inclusions. This test proved serviceable as an indication that the guinea pigs did not carry the submaxillary gland virus.

All of the animals died after injection of the virus. They lived for from 8 to 26 days. The average duration of life for young guinea pigs from stock that carried the virus was 19.5 days, for similar young guinea pigs from unexposed stock 11 days, and for adult animals from the same stock 9.3 days. Autopsies made at the time of death revealed a milder tissue reaction in the animals of Group I than in those of Groups II or III. In the animals of Group I there was found an infiltration of the gland and distention of blood vessels. The number of inclusion bodies in the duct cells was relatively small. In the animals of Groups II and III the glands were hemorrhagic and contained large areas that were completely autolyzed. In regions of severe autolysis the duct cells survived but all of the acinar cells disappeared. Inclusions were numerous in monocytes and in duct cells.

The results indicate that the virus used was sufficiently virulent to kill all animals injected, even those that by association in a colony carrying the disease might be expected, from the experience of other investigators, to withstand the action of the virus. On this basis of comparison it appears that the virus employed was more virulent than that used by other workers. The results also demonstrate that guinea pigs from unexposed stock are more easily affected by the action of the virus than those living in contact with the virus. The differences in susceptibility of the animals in Groups II and III, namely between young and adult guinea pigs of the Genetics stock, are slight, but supported by the additional evidence given in Table III, they may be significant. If so, this indicates that adult animals are more susceptible to the disease than young guinea pigs.

*Correlation of the Number of Inclusions and the Quantity of Virus:* There exists another possible explanation why the virus used in this experiment should appear more virulent than that described previously in the literature. Possibly the animals used as a source of the virus harbored more than the usual amount. Since there exists no direct method for measuring the quantity, the number of inclusion bodies observed was used as a criterion of virus concentration. Numerous investigators have suggested that the inclusion bodies which develop in the duct cells of the submaxillary gland are a fair indication of the presence of the virus, but no effort has as yet been made to correlate the quantity of virus with the number of inclusions.

In Table II, which summarizes the results of the experiment, all guinea pigs that served as a source of virus were shipped from the same dealer but not at the same time. The results obtained indicate how different sources of virus may vary in actual virus concentration. The apparent absence of virus from guinea pigs Nos. 34 to 37, based on the absence of inclusion bodies in 9 sections from a gland of each animal, indicates that failure to find the bodies in a sampled portion of the gland is not complete proof that the virus is lacking. On the other hand, the presence of inclusions is reliable evidence that the virus is present. It is probable that a closer correlation would have been attained between virus concentration and number of inclusion bodies if the number of sections examined had been larger and had been taken from several regions throughout the gland.

Scott<sup>3</sup> is the only investigator who has given data that permit a comparison of results. He injected virus from glands that contained approximately 50 inclusion bodies per section, and although he augmented the pathogenic action of the virus by inoculation of pilocarpine, his animals survived for the duration of the experiment (15 days), whereas those in Groups I and II in Table II, which averaged 39 inclusion-bearing cells per section, died on an average of 7 and 10.4 days respectively. None of the animals survived 15 days.

The reaction of animals in Group IV confirms the conclusion that the virus was more virulent than that employed by Scott; the results obtained with our virus duplicate results obtained by Scott when the concentration of the virus was so low that no inclusion

TABLE II  
Relation between the Number of Inclusion-Bearing Cells in the Submaxillary Gland and the Potency of the Virus

Group	Source of virus			Injection in uninfected guinea pigs		Duration of life after injection	
	Number of guinea pig	Number of inclusions per section	Dilution	Number of animals used	Region of injection	Variation	Average
I	41	(18)*	1:10	4	Submaxillary gland Brain	days 6 to 8 4 to 5	7
	42	91		2			4.5
	43	24					
II	44	20	1:10	6	Submaxillary gland Brain	8.5 to 14 4.5	10.4
	45	60		2			4.5
	46	23					
III	72	14	1:10	3	Submaxillary { 1 animal Brain                    2 animals	14 Recovered 8	8
	73	0		3			
	74	0					
IV	34	0	1:10	14	Submaxillary gland Brain	All recovered 8 to 11	9.3
	35	0		4			
	36	0					
	37	0					

\* Figure obtained after gross correction had been made so that the size would approximate those that follow.



bodies were observed in the sample sections. Evidence that the virus was present in the animals used as the source of virus in Group IV is proved by the reaction of the 4 guinea pigs which were inoculated intracerebrally.

*Dilution Experiments:* Since in the previous experiments gland suspensions of high concentrations were employed, it seemed worth while to investigate the action of a diluted virus.

TABLE III  
*Relation of Virus Dilution to Pathogenesis*

Region of inoculation	Number of guinea pig	Dilution of gland (virus) in Locke's solution	Survival after injection		Presence of inclusions in fixed and stained slides	
			Young guinea pigs	Adult guinea pigs	Brain (monocytes)	Submaxillary gland
Cerebral cortex. Dose 0.05 cc.			days	days		
	120	1:20	6		+	
	124	1:40	9		++	
	129	1:80		7	++	
	127	1:80	Survived			-
	130	1:160	16		-	+
	134	1:320		7	++	
	133	1:320	29*			+
	132	1:320	Survived			-
Subcutaneous, in region of submaxillary gland. Dose, 1 cc. (divided between right and left sides).	122	1:20		10		+++
	123	1:20	9			+++
	121	1:20	24†			+
	125	1:40	10			+++
	126	1:40	12			+++
	128	1:80	19			+++
	131	1:160	31*			

\* May have died from some cause other than direct action of the virus. Autopsy showed an enteritis.

† Sacrificed on the 24th day; apparently normal at this time.

The data are not adequate enough to determine a sharp endpoint in the lethal effects of the virus but indicate that for both intracranial and gland injections of young guinea pigs the endpoint is between 1:80 and 1:160. Submaxillary glands pooled from 6 guinea pigs previously injected once or twice in the course of earlier experiments and which had not died as a result of the injections were used as the source of virus. To these were added the glands from an adult guinea pig purchased from the dealer who supplied the guinea pigs harboring the original strain of virus. Emulsions of gland were diluted from 1:20 to 1:320 (Table III). All guinea pigs injected came from the Genetics stock; most of

them were 3 to 5 weeks old and 3 adult animals were also available. Of the 15 guinea pigs, 8 were injected intracerebrally and 7 subcutaneously in the region of the submaxillary glands. In this experiment pieces of gland tissue were not removed prior to the injection, but the subsequent results leave little doubt that the animals were not carrying the virus.

A recent investigation by Markham<sup>6</sup> on the pathogenicity of various dilutions of the virus makes possible a comparison between two sources of virus. He used intracerebral injections in young guinea pigs, and at dilutions of 1:10 the time of death for 4 animals averaged 7.2 days, and no deaths occurred at a dilution of 1:100 or 1:1000.

The data given in Table III lend support to the results reported in Table I, namely that adult guinea pigs may be more susceptible than young guinea pigs as a dilution as high as 1:320 proved fatal in 1 adult animal following intracerebral injection, but in young guinea pigs and at dilutions of from 1:80 to 1:320 death occurred in only 2 of the 4 animals tested.\*

*Visceral Susceptibility:* Thus far the discussion of lethal effects of the virus has concerned inoculations into the brain and submaxillary regions. In the following experiment injections were made into the testicle, kidney, ovary, spleen, liver and abdominal cavity (Table IV). Other animals inoculated intracranially served as controls against which the lethal effects of the virus could be measured.

Glands from 7 guinea pigs furnished the material for injection; 1 animal came from a dealer and the remaining 6 were of the Genetics stock, which were part of the 14 animals included in Group IV, Table II. All animals had recovered from visible effects of the injections made 41 days earlier. For 8 days preceding the time when the animals were to be sacrificed they received subcutaneously 11 injections of pilocarpine hydrochloride; the dosage was gradually increased during the period from 0.4 to 0.7 mg.

As shown in Table IV, the virus was fatal for every animal regardless of the region in which the injection was made. The

\* Since these experiments were completed another series of inoculations has been made using animals of various weights and ages and, as before, the full grown animals proved to be more susceptible to the action of the virus than the young animals.

time of death varied from 6 to 11 days. The average duration of life (8.2 days) was somewhat longer than that of 6 days which followed intracranial injection. These results, when compared with those of Groups I and II, Table II, seem to indicate that the visceral organs possess about the same degree of susceptibility to virus action as tested by death dealing effects as that found for the salivary gland.

TABLE IV

*Virulence of Virus when Injected in Visceral Organs of Guinea Pig*

Animal number	Age	Quantity of 1:10 dilution virus injected	Organ or region injected	Duration of life after injection
	<i>wks.</i>			<i>days</i>
135	Adult	0.5 cc. in each	Testicle	7
137	Adult	{ 0.2 cc. 0.1 cc.	Kidney Ovary }	6
138	7	{ 0.2 cc. 0.1 cc. 0.05 cc.	Kidney Ovary Suprarenal }	7
140	5	No record	Spleen	7
144	7	1.0 cc.	Liver	11
145	6	1.0 cc.	Liver	8
143	Adult	1.0 cc.	Intraperitoneal	10
141	6	1.0 cc.	Intraperitoneal	11
142	7	1.0 cc.	Intraperitoneal	7
				av. 8.2
146	5	0.05 cc.	Brain cortex	7
150	6	0.05 cc.	Brain cortex	6
151	7	0.05 cc.	Brain cortex	5
				av. 6

From the limited data available there is no evidence that one portal of entry among the abdominal organs offers greater resistance to the spread and action of the virus than any other.

As a secondary problem in connection with this experiment, the effect of pilocarpine proved interesting in that there was no apparent increase in the virulence of the virus or the number of inclusion bodies. Scott<sup>3</sup> found that the number of inclusions increased from 50 to as many as 312 per microscopic field, with an average of 203. There was, however, this difference in his procedure and ours; his treatment with pilocarpine began close to the time of the virus inoculation while ours was done after recovery was complete. The different results obtained by these two methods of

treatment confirm his opinion that stimulation of the gland while the virus is in action influences the susceptibility of the cells. In addition, it also indicates that the persistence of infection is probably not due to reinfection.

*Brain to Brain Passage:* Another minor problem of this experiment was the effort to make brain to brain passages as tried by Cole and Kuttner,<sup>1</sup> Andrewes,<sup>4</sup> and Hudson and Markham.<sup>12</sup> It was thought that the greater virulence of the virus with which we were working might give results different from those reported in the literature. Portions of brains from 3 guinea pigs that died of the disease on the 8th day were inoculated intracranially into 3 adult guinea pigs. All 3 animals survived and were reinoculated intracranially after 17 days with the same virus used for the animals in Group IV, Table II. All survived the reinoculation and so probably had received sufficient virus in their previous intracranial inoculation to confer immunity. These results indicate that the virulent virus behaves in the same manner as the viruses of less pathogenic effects, as reported by other investigators.

### OBSERVATIONS

*Symptoms:* The behavior of the animals following intracerebral inoculation was different from that observed after inoculations made into the visceral or into the submaxillary gland regions. Acute intracerebral infections, which were fatal in 5 to 8 days, usually presented certain nervous reactions such as increased salivation, staggering, loss of equilibrium, incoordination of movements, weakness, or even paralysis of the posterior extremities. Certain animals showed pedaling or convulsive movements prior to death. The variations in temperature were similar to those described by Cole and Kuttner,<sup>1</sup> and Markham<sup>6</sup> in that following injection of the virus there was a protracted high temperature which dropped to a subnormal level about 24 hours before death. Acute infections after inoculation of the virus into the submaxillary gland, the adjacent region, or into the peritoneal cavity or the abdominal viscera, produced death in from 7 to 12 days but failed to elicit nervous symptoms. The usual reaction was that of general depression and loss of appetite after 4 to 6 days. In this condition the animal was not alert, its eyes were often closed, and there was frequently a marked swelling of the submaxillary region

which in some cases seriously impaired respiration. The temperature in different animals was somewhat elevated but never reached the levels caused by the intracerebral inoculations. Death ensued in these animals rather quickly and unexpectedly in comparison with the animals inoculated intracerebrally.

*Histopathology:* The lesions produced by intracerebral inoculation of the virus were similar to those described by many investigators. They consisted of a peracute to acute inflammation of the meninges characterized by extensive hyperemia and monocytosis. The peracute lesions were encountered in the animals that died in from 4 to 6 days after inoculation and consisted of invasion by considerable numbers of macrophages with formation of large giant cells. When death occurred after 6 days there was a decrease in congestive changes but an increase in the number of infiltrating monocytes. There was present also an increasing microglial proliferation in regions of the brain cortex adjacent to the acutely inflamed regions of the meninges. This microglial proliferation was first noted on the 7th day, becoming pronounced on the 9th to 11th days.

Inoculations of the virus into the submaxillary gland or adjacent subcutaneous tissue produced extensive and macroscopically congestive, hemorrhagic and degenerative changes in the whole region. Microscopically there was abundant infiltration with monocytes accompanied by hyperemia, extensive extravascular accumulations of blood, and marked tissue destruction. In addition, macrophages and small giant cells were seen, but to a lesser extent than in the more acute brain lesions already described. Destruction was especially marked in the submaxillary gland tissue. The first gland cells to be destroyed were those of the acini, while the duct cells seemed to be more resistant and frequently persisted in regions where all of the surrounding cells had been completely autolyzed. If the animal survived an acute attack the severely damaged gland regenerated and mitotic figures could be found. Regeneration was almost complete by the 21st day after infection.

Virus inoculated into the visceral organs produced inflammatory reactions similar to those seen in the submaxillary gland. There was extensive degeneration of the inoculated organ, frequently to such an extent that it was difficult to distinguish the original cells

from those that had migrated into the organ, which were mostly monocytes. In addition to these changes there was usually a more or less severe inflammation of the peritoneal cavity. Giant cells were usually found in most of the visceral organs studied.

After inoculation of virus into one part of the body there frequently occurred a reaction in organs distant from the site of injection, for example an injection into the brain of an animal caused a reaction in the lymph glands of the submaxillary region after 5 days, and 8 to 12 days following an injection into the submaxillary gland an inflammatory reaction was present in the spleen and other visceral organs. The reaction in the secondary location was always less severe than that at the original site of inoculation.

*Cytopathology:* The main pathological change observed was the formation of inclusion bodies. In addition to descriptions of inclusion formation in the submaxillary duct and acinar cells the literature includes the following references on inclusion formation: cells of the testis, tongue, and lung (Cole and Kuttner<sup>1</sup>); mesodermal (mesenchymal) cells of the guinea pig fetus (Markham and Hudson<sup>13</sup>); and fibroblasts, endothelial cells, smooth muscle and kidney cells (Pearson,<sup>14</sup> and Kuttner and T'ung<sup>5</sup>).

Inclusions were usually formed at the site of inoculation of the virus, although they were also found frequently in tissues distant from the area inoculated. Of the latter tissues, the submaxillary duct cell was more frequently affected, but the liver cells also tended to develop inclusions, although of a type different from those formed in the gland.

The cells affected directly through local administration of the virus included monocytes, macrophages, endothelial cells and lymphocytes of the spleen and lymph glands, cells of the tubules of the kidney, the thyroid gland, a few nerve cells of the brain cortex, the theca cells of the ovary, interstitial and possibly some germinal cells of the testicle, epithelial cells of the epididymis, and a few cells of the adrenal. The lung, pancreas and mammary glands were not studied. The monocytes constituted a particularly interesting aspect of the disease in that they increased greatly in numbers in whatever tissue was inoculated, infiltrating not only at the site of inoculation but many other distant areas. The monocytes carried inclusion bodies through the course of the clinical

disease up to the 11th day in animals inoculated intracerebrally and up to the 14th day after inoculation in the submaxillary gland.

The wide distribution of these inclusion-bearing cells distant from the site of inoculation is given in Table V, but whether the inclusion is a sign of the presence of virus in all the tissues of the guinea pig during the 5 to 12 days of the disease, or whether it represents a migration of monocytes already carrying the inclusions could not be definitely stated from our experiments until further relations are established between the virus and inclusion-bearing monocytes. Kuttner and T'ung,<sup>5</sup> however, have shown

TABLE V  
*Occurrence of Inclusion-Bearing Monocytes in Regions Distant from Point of Injection*

Animal number	Region inoculated	Death and removal of tissues	Organs with inclusion-bearing monocytes
		<i>days</i>	
52	Submaxillary	8	Spleen, liver
54	Submaxillary	11	Kidney, suprarenal
122	Submaxillary	10	Liver
126	Submaxillary	12	Liver
140	Spleen	7	Submaxillary gland, liver, stomach
144	Abdominal cavity	11	Submaxillary gland?
147	Brain	5	Lymph gland (submaxillary area)
150	Brain	6	Lymph gland (submaxillary area)

that at the 10th day following intracerebral inoculation the virus is present in many tissues of the body.

The evidence seems to indicate that the inclusion-bearing monocyte is not a criterion of the presence or amount of the living virus in that inoculation of large numbers of such cells in brain to brain passages and the tissue culture experiments of Andrewes<sup>4</sup> have failed to keep the virus alive. Andrewes came to the conclusion that in tissue culture survival of the virus is established but multiplication does not occur. On the other hand, the monocyte may possibly engulf and thereby destroy the virus.

## II. CYTOLOGY OF INCLUSION BODY FORMATION

This study of inclusion body formation in the guinea pig and a similar study undertaken on the cytology of fox encephalitis have indicated that the response of the chromatin is a more reliable measure of the age of the inclusion than is the size of the

inclusion body. Intranuclear inclusion bodies must increase in size from a small beginning, but it would seem that this growth is not uniform if compared with changes that occur in the nucleus.

*Development of Inclusion Bodies in Monocytes,  
Macrophages and Giant Cells*

The "early" stage of inclusion formation, judged by the criterion of nuclear reactions, is a slight pulling away from the inclusion body of the chromatin granules and their connecting linin network. In the earliest stages no appreciable halo exists between the inclusion body and the chromatin granules. This condition is rare and usually a halo has developed, as shown in Figures 1 and 2. In both figures the nuclear reaction has progressed to about the same degree in relation to the inclusions. The condition in Figure 1 is more typical in that the inclusion body is single and small. In Figure 2 an extreme variation from the typical condition is illustrated.

Continued margination results in retraction of the chromatin granules toward the nuclear membrane (Fig. 3). As a rule the granules of chromatin which have not yet reached the margin appear still definitely discrete and separate, as do the granules of chromatin in the earlier stages, or as those in the normal resting nucleus. Probably the only pronounced difference is an increase in amount of oxychromatin in the inclusion-bearing cell.\*

As margination proceeds the chromatin which reaches the nuclear membrane first fuses into a seemingly homogeneous mass. Upon this lie groups of chromatin granules which have aggregated together in irregular clumps (Fig. 4). The chromatin in contact with the nuclear membrane becomes inconspicuous but apparently does not diminish in amount. Such a stage as this has been designated as "intermediate." The inclusion body in Figure 4 is

\* Oxychromatin is a term difficult to define because we have little definite knowledge concerning its chemical nature. In stained preparations it is usually regarded as an acidophilic substance. It has a negative Feulgen reaction. There seem to be two types of acidophilic material in the nucleus: first, a portion that behaves like the basichromatin, in that it mingles with it, has nearly the same specific gravity, and is readily pulled away from the nuclear membrane during centrifugation; and second, a portion of viscous nature which during centrifugation stretches into fine strands when pulled by the heavier parts of the nucleus. Wilson<sup>15</sup> is of the opinion that oxychromatin and basichromatin are two phases of the same substance.



about as large as that in Figure 7, which illustrates again how variable their size may be in relation to the nuclear reaction.

In the particular cell from which Figure 4 was made the inclusion body revealed two parts which, structurally and by the staining reactions, are distinct from each other. One is a spherical portion with an affinity for acid dyes, such as eosin used in the hematoxylin and eosin stain or in Giemsa's stain. This body is relatively homogeneous in structure. The second is a darker staining portion which usually contains spherical vacuoles. The usual relationship of the two portions is shown in Figures 4 and 6, in which the darker vacuolated mass forms a cap at one side of the acidophilic body, but occasionally the bodies are completely separated (Fig. 5 ABIN and AIN). This latter arrangement emphasizes the fact that the bodies are not merely the result of an optical phenomenon. However, evidence from staining procedures makes it seem probable that both bodies are of similar origin, although they stain differently. The darker body is not colored with the hematoxylin in the same way as is chromatin, instead it appears gray or slate colored. Feulgen's reaction alone does not color the acidophilic body but does color lightly the "dark body." When the Feulgen reaction with a light green counter stain is employed the acidophilic body stains green and the "dark body" stains gray or grayish blue. It is interpreted from these reactions that the dark inclusion body is fundamentally similar to the acidophilic body and that it has an affinity for the light green stain to the same extent, but in it there is dissolved chromatin, which gives it its slightly positive Feulgen character; the red and green dyes mixed give the slate color. Likewise, the combination of blue and orange colors from the hematoxylin and eosin stain gives a bluish slate color. In order to have a term for each type, one has been called the acidophilic inclusion and the other the acidobasophilic inclusion. Why one inclusion body should have an affinity for chromatin and the other should not finds no conclusive answer at present. Some meager evidence points to a difference in age, the one that contains chromatin being the older. Reason for this suggestion comes from the basophilia observed in many inclusion bodies under conditions when they are known to be old. Inclusions of both types are found not only in monocytes but also in young intranuclear inclusions of the submaxillary gland ducts.

The process of margination of the nuclear chromatin continues from that represented by Figure 4 and relatively few separate granules remain still distinguishable, a late "intermediate" state (Fig. 5). Margination ultimately goes to completion (Figs. 6, 7 and 8) which has been designated as a "late" state. The nucleolus may lag behind in this process but later reaches the nuclear membrane (compare Figures 6 and 8). At this stage it was expected from a previous study on fox encephalitis that the margined chromatin would break up and aggregate into masses, but this was not the case with the monocytic intranuclear inclusions of the submaxillary gland virus of the guinea pig. In studying the final fate of these cells only those that were surrounded by living and unautolyzed cells were considered in order to eliminate, if possible, all non-specific extracellular toxic effects as indirect causes of cell changes. The number of cells which fulfilled this precautionary measure was relatively few. Only very rarely was autolysis seen in monocytes located in the midst of living cells. The process of autolysis when observed is similar to that found in giant cells (Fig. 10); the chromatin liquefies, spreads throughout the nucleus and obscures the inclusion body. Nuclear fluids disappear and ultimately the nuclear membrane shrinks against the inclusion body. This type of autolysis is similar to that observed by Lucas and Cowdry in the nuclei of liver cells in healthy rats. The rats were killed by a blow on the head and their bodies put in the incubator at 37° C. for various periods of time. The condition in Figure 10 is somewhat similar to that shown by the nuclei of a rat's liver after the animal had been dead for about 18 hours.

Since so few autolyzing monocytes were found in the meninges of the brain, search was made among other tissues, such as the spleen, cervical lymph glands and several visceral organs, to find, if possible, where these autolyzing cells were more abundant. They were not present in any of these tissues in numbers sufficient to make it seem likely that their fate was the simple autolysis for which we had been looking. Attention was drawn ultimately to the giant cells. In the literature has been described the transformation of monocytes and macrophages into giant cells. There was evidence that these two cell types were probably involved in the formation of giant cells in our material. Autolysis of giant cells

was found more frequently than the autolysis of monocytes and it was concluded, therefore, that some monocytes, at least, undergo autolysis after they become giant cells rather than in the mononuclear condition.

As far as we are aware this is the first time that the occurrence of giant cells has been noted after the injection of submaxillary gland virus. They do not appear in every preparation where there is an infiltration of monocytes. They may be found as early as 4.5 days, as well as in later stages when the acute congestive changes have subsided.

Classification of giant cells, particularly the separation of epithelioid giant cells (Langhans' giant cell) and foreign body giant cells is difficult without the use of vital dyes. Doan, Sabin and Forkner,<sup>16</sup> and Forkner<sup>17</sup> noted a structural difference, however, in that the nuclei of the epithelioid giant cell are arranged at the periphery of the cell, whereas the foreign body giant cell has its nuclei scattered through the cytosome. Another distinction for the separation of the two types is that the epithelioid giant cells are thought to be formed by amitotic divisions of monocytes, whereas foreign body giant cells form from the fusion of macrophages.

Both types of giant cells were frequently found in our material but the epithelioid giant cell was perhaps more in evidence because the arrangement of its nuclei made it more conspicuous (Figs. 10 and 11). Lobulation of nuclei was more abundant in smaller than in larger cells. There were many cells with deep constrictions among the lobes which presumably were in the process of dividing into separate nuclei. In many cases two large lobes would be held together only by a small strand. This type of lobulation was observed by Unna,<sup>18</sup> Tyzzer,<sup>19</sup> and Lipschütz<sup>20</sup> following infection with herpes, varicella and variola, and vaccinia, and has been called by these authors "ballooning degeneration," the end result of which is the production of a cluster of nuclei in the center of the cell.

Foreign body giant cells were identified by their scattered nuclei and the remains of phagocytosed debris in the cytosome. Whether fusion of cells which is characteristic of foreign body giant cells occurred in our material is difficult to establish with certainty. There were frequently observed, however, cells pressed into the

side of a giant cell, and in some cases part of the cell wall between the two seemed to be absent.

Certain small giant cells seemed to take their origin from the endothelium of blood vessels in congested regions and they were found in numbers within blood vessels.

Although there may be found both types of giant cells, yet there are all intermediate grades so that it is not certain to what extent we are dealing with valid foreign body giant cells or with epithelioid giant cells in which the monocytes from which they were formed were modified as described by Carrel and Ebeling,<sup>21</sup> and Lewis,<sup>22</sup> in the transformation of monocytes to macrophages.

If giant cell formation represents the ultimate fate of a portion of the monocytes with inclusions, and since autolysis is frequently observed in these cells, it may explain the rarity of autolysis among mononuclear monocytes. The process of autolysis in the nuclei of giant cells is similar to that for the monocyte, namely liquefaction of the chromatin, loss of nuclear substance, and shrinkage of the nuclear membrane (Fig. 10).

In some of the giant cells there were nuclei without inclusion bodies or the inclusions present were in different stages of development, but in the majority of cases all of the nuclei in one giant cell were in the same stage of development, namely, complete margination. There is the possibility that the giant cells and monocytes do not always deteriorate and die as a direct result of their abnormal states; Forkner,<sup>17</sup> for example, is of the opinion that giant cells may later split up into component cells but there has been no evidence for such a process in our material.

Whether the inclusion bodies of monocytes or giant cells always undergo autolysis or persist after the cell disintegrates has an important bearing on the relation of inclusion bodies to the virus agent. The inclusions in Figure 10 would seem to show disintegrative changes as described by Ivanovics and Hyde<sup>23</sup> for virus III in tissue culture. On the other hand, old inclusions entirely free from their cells could be definitely identified in the intercellular spaces and such bodies have been phagocytosed by other cells (Fig. 8 ININ).

The time involved in the various phases of the process of inclusion formation and development in the monocyte is not known since all stages appear simultaneously in one preparation. The

relative time may however be estimated from the relative abundance of cells in different development stages. Early stages (Figs. 1, 2 and 3) and autolytic stages (Fig. 10) are few in comparison with the intermediate and late stages (Figs. 4, 6, 9 and 11) which seems to indicate that early and autolytic stages probably change rapidly and that intermediate and late stages are more enduring. It is also probable that the whole cycle of inclusion-bearing monocytes is relatively short in comparison with the life of infected duct cells of the salivary gland since the former shows late stages and autolysis at the end of 4 or 5 days, whereas development of late stages in the latter requires about 2 weeks.

*Development of Inclusions in Duct Cells of the  
Submaxillary Gland*

A reconstruction of the sequence of events in the duct cells is attended with much greater certainty than in the monocytes because the development of inclusion bodies in the duct cells is synchronized. Because of this it is possible to gain a fairly accurate estimate of the time required for the development of inclusion bodies "*in vivo*."

Kuttner,<sup>2</sup> Andrewes,<sup>4</sup> Kuttner and T'ung,<sup>5</sup> and Hudson and Markham<sup>12</sup> noted that the development of inclusion bodies was usually delayed until about the 10th to 15th day after inoculation. The occurrence of an initial delay has been observed in our material except that inclusions appeared earlier. They were never absent in any animal that died later than the 6th day.

The nuclear chromatin reacts to the presence of a newly formed inclusion body in about the same way as in the monocyte, in that there is a drawing of the chromatin away from the inclusion body. At first this action is slight (Fig. 16, 7 days, and Fig. 17, 11 days). In the "early" stages strands of oxychromatin still cross the developing halo and the chromatin granules are separate and discrete. The process of margination goes approximately to completion (Fig. 18, at the 11th day) which is still considered as an early stage in the formation of inclusions in the duct cell whereas it would be considered "intermediate" to "late" in the monocyte. It should be emphasized that usually more than one stage of development as given in the figures could be found on a single slide, but there were definite limits to this variation so that the

stages of development could be fairly closely correlated with the length of time the animal survived after inoculation.

Chromatin margination is similar to that observed in the monocytes, but in the latter autolysis seems to be the next stage in the cycle, whereas in the duct cell a different reaction occurs. The chromatin, after it attains the margin, or even before that stage is reached (Fig. 19, 13 days, and Fig. 22, 17 days) breaks away and moves toward the inclusion body. This is considered as the "intermediate" stage in nuclear reaction; it includes the period when the amount of centrally migrated chromatin is still small and particulate, and more or less uniformly distributed around the inclusion body. With increasing age more of the chromatin accumulates (Fig. 23, 17 days, and Fig. 20, 21 days). Then follows a slight clumping of chromatin granules which ultimately goes as far as illustrated in Figure 21, a cell taken from a section of tissue from a stock animal used as a source of virus. The clumping of the chromatin, which has moved centrally, and its almost complete absence on the margin is designated as an "old" stage. In none of the cells observed, however, were all the granules clumped together. As a result of the central migration of chromatin the nuclear margin becomes nearly bare.

The description of the old stages agrees with the previous studies made by one of us,<sup>8</sup> with the exception of one point. In the experiments published in 1936 it was stated that all of the basichromatin moved centrally. This appeared to be the case when hematoxylin and eosin were used as a stain, but after the Feulgen reaction was applied (Figs. 22, 23 and 24) it was discovered that a small amount of finely dispersed basichromatin remained mingled with the oxychromatin on the nuclear membrane.

When these criteria of age are applied to the colored illustrations of submaxillary gland duct cells by Cole and Kuttner,<sup>1</sup> Figure 2 would be regarded as a young stage, as they suggested, and Figure 1 an intermediate stage.

The final fate of inclusion-bearing cells could not be established definitely. In many cases they were apparently extruded into the lumens of the ducts and this was observed at various stages of inclusion formation. Autolysis was observed in only a few of the older cells, but this occurred only in regions where tissue destruc-

tion was pronounced. When it did occur it followed the same cycle as revealed in degenerating monocytes, namely liquefaction of the chromatin, loss of nuclear fluids and shrinkage of the nuclear membrane against the inclusion body (Fig. 25).

A characteristic cellular reaction recorded in most of the literature and also found in our studies is the occurrence of cell and nuclear hypertrophy. The cell in Figure 18 (11 days after inoculation) already shows marked hypertrophy. This effect is continued for at least 2 weeks after the first appearance of intranuclear inclusions (compare Fig. 18 with Fig. 24 from an animal 24 days after inoculation).

The inclusion body during its formation often shows the same two types of parts found in monocytes; one stains gray (acidobasophilic inclusion) with hematoxylin and eosin, and the other pink (acidophilic inclusion). The same explanation of formation holds true, namely that the gray body contains a certain amount of dissolved chromatin. As the inclusions become older the amount of dissolved chromatin increases and old inclusions nearly always stain darkly.

The cytoplasmic inclusions were probably first observed by Jackson<sup>24</sup> who reported them as parasitic organisms. Pearson<sup>14</sup> and Andrewes<sup>4</sup> later studied them. Andrewes considered them secondary to nuclear inclusions, while Pearson proved them to be faintly Feulgen-positive, a fact later confirmed by Markham.<sup>6</sup> Similar bodies have been observed in the submaxillary glands of man by Goodpasture and Talbot,<sup>25</sup> and by Farber and Wolbach.<sup>26</sup>

In our study the cytoplasmic inclusions are scarcely visible when stained with hematoxylin and eosin after fixation in Zenker's acetic acid solution, while the stain is somewhat better after fixation in corrosive sublimate plus acetic acid. They stain blue with Giemsa's stain. The results are best after the Feulgen reaction, by which method the inclusions are the only cytoplasmic elements that give a positive reaction. The cytoplasmic inclusions proved to be different from the intranuclear ones in that they had an affinity only toward the Feulgen reaction and not toward the light green counterstain which indicated a distinct difference between the two inclusion bodies. The Feulgen-positive character of the intranuclear inclusion is a secondary quality, whereas it is a primary one for the cytoplasmic inclusion. Haagen<sup>27</sup> reports that

the cytoplasmic inclusion of vaccinia virus is Feulgen-positive but is of the opinion that in this case the Feulgen-positive material is derived from the basichromatin of the nucleus.

The development of cytoplasmic inclusions lags behind that of nuclear inclusions, as already reported by Andrewes.<sup>4</sup> They appear on or before the 17th day after inoculation (Fig. 22), which is 6 to 10 days after the first appearance of the intranuclear inclusions. The cytoplasmic inclusion appears first as a small granular body, but in the same slide from which Figure 22 was chosen, older stages were found in which the number and size of these bodies had increased, as shown in Figure 23 *cn*. In this particular cell the particles which aggregated to form the inclusion bodies were sufficiently separated so they could be distinguished individually. It proved difficult to measure the diameter of a single particle because Feulgen's technique does not stain very strongly a particle so small, but their diameters were estimated to be about 400  $\mu\mu$ . When the inclusion bodies are older they form compact masses and individual particles are no longer visible (Fig. 24, 24 days), and the number of inclusion bodies increases also.

When these cells autolyze (Fig. 25) the outline of the cytoplasmic inclusions becomes less definite, but the reaction may be due more to disruptive and disintegrative changes in the cytoplasm than to autolysis within the inclusion body itself.

These cytoplasmic inclusions are usually observed at the lumen side of the cell, which usually presents the larger amount of cytoplasm. This agrees with observations by Pearson.<sup>14</sup>

The rate at which intranuclear and cytoplasmic inclusion formation takes place may be summarized as follows: intranuclear inclusions may appear as early as the 6th day after inoculation but probably they develop later in the animals less acutely affected. The early steps of margination proceed rapidly and are usually found with the beginning stages. The migration of the chromatin toward the inclusions (intermediate stage) begins about the 13th day after inoculation and extends up to the 17th day. The old stages, characteristic of the condition found in stock animals, appear on the 21st day after inoculation and are abundant by 24 days.

The cytoplasmic inclusions first appear when the nuclear cycle is half completed, namely about 17 days. They develop more



rapidly than the nuclear stages so that at 24 days they are as numerous as they are in stock animals.

It has been mentioned that intranuclear inclusions develop in many other tissues of the body, but the sections prepared were not sufficiently abundant to allow an accurate determination of the sequence of developmental stages. Those in the liver cells were given particular attention because of their similarity to the "chemical inclusions" reported by Olitsky and Harford.<sup>28</sup> \* The inclusion body in the liver cells is hyaline, stains with acid dyes, although rather lightly, and the particular feature which made them appear similar to those reported by Olitsky and Harford is the apparent compatibility of the chromatin with the inclusion body and the absence of a clearly defined halo around the inclusion body (Fig. 15). The chromatin in the cells is only very rarely completely margined; usually there is little or no margination. The nuclear reaction in the liver cells is similar also to that illustrated and described by Blackman<sup>29</sup> in kidney cells following lead poisoning.

### *Effects of Ultracentrifugation on Inclusion-Bearing Cells*

Earlier studies on the centrifugation of herpes inclusions in cells of the rabbit cornea demonstrated that the inclusion body was lighter than either the nuclear sap or the chromatin.<sup>7</sup> The centrifugation of glands from stock guinea pigs infected with the submaxillary virus failed to separate the chromatin from the intranuclear inclusion and both were carried to the centrifugal pole.<sup>8</sup> It was hoped that by using early stages before the chromatin joined the inclusion body the relative specific gravity of the inclusion body could be determined and compared with that of herpes.

In the present investigations the effects of centrifugation on duct cells of different ages, on monocytes and on lymphocytes were studied. The movement of the chromatin in both lymphocytes (Fig. 12) and monocytes (Figs. 13 and 14) is independent of the degree of margination. The partially or totally margined chromatin moves to the centrifugal pole while the inclusion body remains suspended in the nuclear sap. When completely margined the chromatin seems to slide along the nuclear membrane

\* Dr. Olitsky and Dr. Harford kindly sent us a stained slide and a block of tissue from their experiments in order that we might make more direct comparisons.

to reach the centrifugal pole. The number of cells available for study were too few to give any idea whether the inclusion body, if centrifuged longer, would have moved upward or downward through the nuclear sap in which it was suspended. In several cells two types of intranuclear inclusions were distinguishable. After centrifugation (Fig. 13), however, no consistent orientation could be observed which would indicate that one was heavier than the other.

In the submaxillary gland duct cells, 10 days after inoculation when the intranuclear inclusion bodies are beginning to form and when little or no chromatin has moved on to the inclusion body, centrifugation concentrated the basichromatin and oxychromatin at the centrifugal pole (Fig. 26). The inclusion body follows the chromatin, and thus demonstrates that it is lighter than the chromatin but heavier than the nucleoplasm.

When the stage of development has been reached in which the chromatin particles migrate to the inclusion body, centrifugation carries the loose marginated chromatin to the centrifugal pole but it does not separate that which has joined the inclusion body (Fig. 27, 14 days).

By the time cytoplasmic inclusions are numerous and the nuclear changes are well advanced, centrifugation causes the movement of the inclusion and its adherent chromatin to the centrifugal pole where it may produce a bulging of the nuclear membrane, as in Figure 28. In addition, the evidence is fairly conclusive that the cytoplasmic inclusions are heavier than anything else in the cytosome, and perhaps as heavy as the nucleus. In nearly every cell studied they were located as close to the centrifugal pole of the cell as the nucleus would permit. In some cases the nucleus and its inclusion body touched the cell wall and under these conditions the cytoplasmic inclusions were located at the side of the nucleus, but near the centrifugal pole. The high relative specific gravity of the cytoplasmic inclusion perhaps has some relation to the Feulgen-positive nature of the material of which it is composed.

### III. DISCUSSION

Many points of limited interest were discussed under the experimental data. Several of the larger problems on which these

investigations have a bearing are the origin and fate of inclusion bodies, and the viability of the cells which bear them. The significance of the centrifugation studies will be discussed.

### *Origin and Fate of Inclusion Bodies*

Nearly all structural parts of the cells have been suggested as the source or origin of inclusion bodies. In addition they have been considered to be aggregations of virus particles. As an example, Negri bodies have been suggested to be of neurofibrillar and mitochondrial origin by Goodpasture,<sup>30</sup> from Nissl substance by Covell and Danks,<sup>31</sup> and Nicolau and Kopciowska.<sup>32</sup> The origin of vaccinia bodies has been stated to be from basophilic material of the cytoplasm (Cowdry<sup>33</sup>), from nuclear material passed from the nucleus to the cytosome (Taniguchi *et al.*,<sup>34</sup> and Haagen<sup>27</sup>), from accumulation of virus elementary bodies (Paschen,<sup>35</sup> Goodpasture, Woodruff and Buddingh,<sup>36</sup> and Burnet and Andrewes<sup>37</sup>). In the formation of intranuclear inclusions it has at times been suggested that the plasmosome hypertrophies to form the acidophilic body. In this regard it is probably worthwhile to present evidence that this is not the case in the inclusion body formation of either the monocyte or of the submaxillary duct cell. First, a nucleolus is absent in some of the blood cells, namely lymphocytes and small monocytes, so that the acidophilic body could not develop from the plasmosome. In large monocytes (Figs. 6, NL and 8), in endothelial cells, and in nerve cells in which inclusion bodies were formed, the nucleolus was pushed toward the nuclear membrane and showed no affinity for the developing inclusion body. The most convincing evidence, though, comes from the submaxillary duct cell (Fig. 23) and the liver cell (Fig. 15 NL). In the duct cells the plasmosome, which is distinguishable during the margination process, is pushed against the nuclear wall. This is shown well also in the liver cell in which the inclusion body is distinct from the plasmosome. The plasmosome is covered with adherent basichromatin, but the inclusion does not attract the basichromatin in the same way.

Related to the problem of lineage of cell organelles in inclusion formation is the problem dealing with the causes underlying margination, separation of oxyphilic and basophilic chromatin, the time it takes place, and the intracellular movements of other cell

material. The mechanics of margination are apparently the same in the three examples studied closely, namely, the meningeal cells reacting to the virus of fox encephalitis, and the monocytes and the duct cells of guinea pigs under the action of the submaxillary gland virus. There is this interesting difference which may have significance as more material becomes available; the inclusion body of fox encephalitis is Feulgen-positive from its earliest beginning, while that of the submaxillary disease is negative in the early stages. Corresponding to these differences, in fox encephalitis the oxychromatin completely separates from the basichromatin and migrates centrally to apply itself to the surface of the inclusion body while the basichromatin moves to the nuclear membrane. In the submaxillary gland duct cells of guinea pigs both oxychromatin and basichromatin marginate, but after the initial margination spurt is over the basichromatin returns to the acidophilic inclusion body and becomes so closely attached that it has not yet been separated by centrifugal forces over 500,000 times gravity. Thus, in 1 case oxychromatin unites with a basophilic inclusion body, and in the other basichromatin unites with an acidophilic inclusion body.

The question might be raised, why does not the same central movement of chromatin take place in the monocytes as described for the duct cells? It does take place, for example, in Figure 7, but it is found only rarely and never to a greater degree than illustrated. Only one suggestion can be offered as to why it does not go as far in the monocyte as it does in the duct cell, namely that the monocyte does not survive long enough. We do not know the life span of the cells in a congested area, but the complete cycle of tissue changes from inoculation to the period when monocytes have reached a peak in numbers is about 5 days, and in the next 5 days practically all have disappeared. The life span of a single cell in such an area of rapid change is either very short or it changes so completely that its lineage has not been followed. If its life were less than from 3 to 5 days, then the central movement of chromatin would not have time to develop, because it has already been shown in the duct cells that the central migration comes after about 5 days.

For a number of years it was felt that the presence of intranuclear inclusion bodies was strong evidence for the existence of

a virus, but that point of view has lost ground by the researches of Luger and Lauda,<sup>38</sup> Lee,<sup>39</sup> Blackman,<sup>29</sup> and recently Olitsky and Harford.<sup>28</sup> These investigators report the formation of inclusions by the use of chemical agents, including metals, such as lead, iron, aluminum and arsenicals. In addition, Olitsky and Harford<sup>40</sup> produced inclusions by the use of virus-free brain tissue.

According to these reports from the literature the question arises, are the intranuclear inclusions accompanying cellular reactions produced in the monocytes of the guinea pig by the chemical methods of Olitsky and Harford identical with those produced by the virus? It has been shown that the cytological picture in the monocytes under the action of the virus is different from the chemical inclusions, but on the other hand the virus has produced a reaction in the nuclei of liver cells which is practically identical with the chemically produced inclusions of the monocytes. The cytology of the kidney and liver nuclei pictured by Blackman after lead poisoning are quite similar to those we find in the liver after inoculation of submaxillary gland virus, and similar to those obtained in monocytes by Olitsky and Harford, and both of these are similar to the earliest stages in nuclear reaction to a virus. A final answer cannot yet be given, but the simplest suggestion based on facts at hand is that different results are a measure of different degrees of severity of action on the cell by the agents employed, namely that the liver cell under virus action, and the monocyte under chemical action, are responding to a mild stimulus, and thus most of the cells reach only an early stage in nuclear reaction, whereas the monocytes and duct cells under virus action are severely affected and go to the full extent of the response.

Our data give us no information as to whether the intranuclear inclusions are aggregates of particles or are only cellular reaction products. Markham<sup>6</sup> is of the opinion from his study on monocytes in the living condition that there are present in the nucleus ". . . units comparable to the elementary bodies found in other virus infections." Our fixed and stained preparations in which the techniques employed were different from his, revealed nothing of this granular nature. The only structures suggestive of elementary bodies in our studies were observed in the cytoplasmic

inclusions of the duct cells. Their size ( $400\mu\mu$ ) is larger however than that given for the elementary bodies of other cytoplasmic inclusions. There are as yet no detailed studies on the size of the submaxillary virus by filtration methods. Cole and Kuttner<sup>1</sup> noted that it was too large to pass a Berkefeld N filter.

### *Viability of an Inclusion-Bearing Cell*

The opinion has sometimes been expressed that a cell which bears an inclusion is dying; on the other hand, we have found records by Ludford and Findlay<sup>41</sup> and Goodpasture<sup>42</sup> that cells which contain cytoplasmic inclusions of fowl pox divide by mitosis. So far, however, there is no record of mitosis in cells that contain intranuclear inclusions, although amitotic division in these cells has been described by Lipschütz<sup>20</sup> in vaccinia, and by Ivanovics and Hyde<sup>23</sup> in tissue cultures infected with virus III. In our studies we have encountered this phenomenon of amitosis in monocytes and macrophages which contained inclusion bodies.

All of this evidence points to a certain degree of vital activity and the cells which are capable of such response are, of course, not dead. Just how soon they are going to die is difficult to determine and it is still more difficult to determine whether the presence of the inclusion body will cause them to die sooner than neighboring cells which lack inclusion bodies. In regard to the viability of the submaxillary duct cell inclusion, the evidence brought out by Pearson<sup>14</sup> and further advanced by Cowdry<sup>43</sup> that these cells represent dead cells in a state of static equilibrium, because of their resistance to peritoneal autolysis, seems to us rather unsatisfactory evidence of death since it might also represent a static condition of life of the cell making it more resistant to autolytic processes in the peritoneal cavity. Pearson<sup>14</sup> has suggested that the absence of mitochondria in the inclusion-bearing duct cells is evidence that they are dead. However, Sabin, Doan and Cunningham<sup>44</sup> observed that macrophages in tissue culture lacked mitochondria and these cells were definitely alive. In our studies we found some regions in which the state of the nuclear reaction to the inclusion had progressed quite a bit further than the usually observed "old" state, and these changes represented distinct autolytic effects (Fig. 25). These zones of autolysis were surrounded

by extensive tissue destruction and the effect may have been due to influences other than the virus; still it represents valuable evidence that the cells can undergo death changes, and when they do die the process is similar to that of other cells. This evidence would therefore place the intranuclear inclusions in the submaxillary duct cells in a "static life state" rather than one of death. The fact that these inclusions are said to persist for long periods of time, which we could verify indirectly by the fact that we never encountered "young" stages of inclusion formation, such as those of Figures 16 to 18, in any of the guinea pigs used as virus sources, brings out further the theory of viability of these cells.

Studies of Autolysis on rats show that at body temperature cells disintegrate in 18 hours after death of the animal, but the inclusion bodies in the duct cells of the guinea pig develop slowly over a period of at least 2 weeks, which fact may be additional evidence that an inclusion-bearing cell is still capable of vital activities. Scott and Pruett<sup>45</sup> in their cytological study of the volume of inclusion bodies present facts which may be interpreted as evidence for the idea that the inclusion-bearing cells are alive. They have given careful measurements of nucleocytoplasmic relations in salivary gland duct cells, with and without inclusion bodies. If one goes a step further with their data and calculates the nucleocytoplasmic relations after subtracting the volume of the inclusion body from the volume of the nucleus, the ratio of nucleus to cytoplasm of infected cells is approximately normal, which indicates that during the great hypertrophy of the nucleus and cytoplasm, physiological ratios characteristic of the normal living cell were maintained.

The viability of inclusion-bearing cells is still further championed by Nicolau and Kopciowska,<sup>46</sup> who report that such cells are not only alive but contain inclusions because they are particularly resistant to the action of the virus. Nicolau, Kopciowska and Mathis<sup>47</sup> go so far as to state that in mice infected with yellow fever inclusions in the brain begin to recede and disappear by the 11th to the 14th day after infection and that the same cells which carried the inclusions eliminated them from the nucleus and returned to a normal condition. We have no definite evidence, however, from our studies to suggest anything of a similar regenerative nature with the submaxillary gland virus.

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### *Viability of an Inclusion-Bearing Cell*

The opinion has sometimes been expressed that a cell which bears an inclusion is dying; on the other hand, we have found records by Ludford and Findlay<sup>41</sup> and Goodpasture<sup>42</sup> that cells which contain cytoplasmic inclusions of fowl pox divide by mitosis. So far, however, there is no record of mitosis in cells that contain intranuclear inclusions, although amitotic division in these cells has been described by Lipschütz<sup>20</sup> in vaccinia, and by Ivanovics and Hyde<sup>23</sup> in tissue cultures infected with virus III. In our studies we have encountered this phenomenon of amitosis in monocytes and macrophages which contained inclusion bodies.

All of this evidence points to a certain degree of vital activity and the cells which are capable of such response are, of course, not dead. Just how soon they are going to die is difficult to determine and it is still more difficult to determine whether the presence of the inclusion body will cause them to die sooner than neighboring cells which lack inclusion bodies. In regard to the viability of the submaxillary duct cell inclusion, the evidence brought out by Pearson<sup>14</sup> and further advanced by Cowdry<sup>43</sup> that these cells represent dead cells in a state of static equilibrium, because of their resistance to peritoneal autolysis, seems to us rather unsatisfactory evidence of death since it might also represent a static condition of life of the cell making it more resistant to autolytic processes in the peritoneal cavity. Pearson<sup>14</sup> has suggested that the absence of mitochondria in the inclusion-bearing duct cells is evidence that they are dead. However, Sabin, Doan and Cunningham<sup>44</sup> observed that macrophages in tissue culture lacked mitochondria and these cells were definitely alive. In our studies we found some regions in which the state of the nuclear reaction to the inclusion had progressed quite a bit further than the usually observed "old" state, and these changes represented distinct autolytic effects (Fig. 25). These zones of autolysis were surrounded



by extensive tissue destruction and the effect may have been due to influences other than the virus; still it represents valuable evidence that the cells can undergo death changes, and when they do die the process is similar to that of other cells. This evidence would therefore place the intranuclear inclusions in the submaxillary duct cells in a "static life state" rather than one of death. The fact that these inclusions are said to persist for long periods of time, which we could verify indirectly by the fact that we never encountered "young" stages of inclusion formation, such as those of Figures 16 to 18, in any of the guinea pigs used as virus sources, brings out further the theory of viability of these cells.

Studies of Autolysis on rats show that at body temperature cells disintegrate in 18 hours after death of the animal, but the inclusion bodies in the duct cells of the guinea pig develop slowly over a period of at least 2 weeks, which fact may be additional evidence that an inclusion-bearing cell is still capable of vital activities. Scott and Pruett<sup>45</sup> in their cytological study of the volume of inclusion bodies present facts which may be interpreted as evidence for the idea that the inclusion-bearing cells are alive. They have given careful measurements of nucleocytoplasmic relations in salivary gland duct cells, with and without inclusion bodies. If one goes a step further with their data and calculates the nucleocytoplasmic relations after subtracting the volume of the inclusion body from the volume of the nucleus, the ratio of nucleus to cytoplasm of infected cells is approximately normal, which indicates that during the great hypertrophy of the nucleus and cytoplasm, physiological ratios characteristic of the normal living cell were maintained.

The viability of inclusion-bearing cells is still further championed by Nicolau and Kopciowska,<sup>46</sup> who report that such cells are not only alive but contain inclusions because they are particularly resistant to the action of the virus. Nicolau, Kopciowska and Mathis<sup>47</sup> go so far as to state that in mice infected with yellow fever inclusions in the brain begin to recede and disappear by the 11th to the 14th day after infection and that the same cells which carried the inclusions eliminated them from the nucleus and returned to a normal condition. We have no definite evidence, however, from our studies to suggest anything of a similar regenerative nature with the submaxillary gland virus.

### *Centrifugation*

Evidence that centrifugation does not kill the cells but instead that tissue cells can withstand centrifugal forces sufficient to accumulate the chromatin at one pole is given by Guyer and Claus,<sup>48</sup> and MacDougald, Beams and King,<sup>49</sup> so that we may assume that the stratification of substances we observe has taken place in a living system and not in cells that were killed sometime prior to the results obtained.

The previous work with herpes inclusions<sup>7</sup> demonstrated that they were lighter than any normal constituents of the nucleus, but in our study on the intranuclear inclusions of duct cells the bodies proved to be heavier than the nuclear sap, but apparently lighter than the chromatin. The same result was observed in fox encephalitis. No generalization can be made until more cells affected by other viruses have been centrifuged but the differences between herpes and other viruses may prove significant in that the herpes inclusions are of the granular particulate type (type A of Cowdry<sup>50</sup>) while those in the monocytes and submaxillary duct cells, as well as those in the meningeal cells of fox encephalitis, under fixed and stained conditions, are a hyaline non-granular type, similar to the "chemical inclusions."

Considerable evidence is available in the work of MacCallum and Oppenheimer<sup>51</sup> and Schlesinger<sup>52</sup> on the absolute specific gravity of various viruses, but these data cannot be utilized in a study of the relation between inclusion bodies and virus until some method is devised to determine the absolute specific gravity values of chromatin, nuclear sap and other cell organelles.

### SUMMARY AND CONCLUSIONS

1. A submaxillary gland virus of guinea pigs was found which proved to be more virulent than that previously reported in the literature in that it produced death regardless of the region in which the inoculation was made, whereas the submaxillary gland virus of guinea pigs usually kills only after intracerebral inoculation.
2. Young virus-free guinea pigs taken from a colony that furnished the virus were slightly more resistant to the action of the virus than guinea pigs of the same age taken from a colony in which all the members were free from the virus.

3. There is some evidence that the young guinea pigs are more resistant to the action of the virus than the full grown animals of the same stock.

4. This virus failed to maintain itself in brain to brain passages which agrees with results obtained when a less virulent type was used.

5. The symptoms and histopathology of the disease follow the same course as described by others for this virus.

6. Giant cells of both the epithelioid and foreign body types have been found in all tissues of the body where inclusion-bearing monocytes may be seen.

7. Intranuclear inclusions have been observed in lymphocytes, monocytes, macrophages, endothelial cells, cells of the tubules of the kidney, follicles of the thyroid, theca cells of the ovary, interstitial and possibly germinal cells of the testicle, epithelial cells of the epididymis, adrenal and a few nerve cells of the brain cortex.

8. Inclusion-bearing monocytes appear in numerous tissues remote from the site of injection.

9. The inclusions are frequently composed of two parts, an acidophilic portion and an acidobasophilic portion.

10. The stages of the nuclear reactions in monocytes to the developing intranuclear inclusions have been divided into young, intermediate and late. The young stages are those in which the halo is small, the chromatin discrete, and the linin network extensive with little or no margination. The intermediate stages are those in which about half of the chromatin has margined, and the rest clumped, but still showing the particulate nature of the material forming the clumps. The late stages are those in which the chromatin forms a layer of uniform thickness. In very late stages a few granules of chromatin may return to the surface of the inclusion body.

11. The ultimate fate of the inclusion-bearing monocyte sometimes is autolysis. More frequently the monocyte is involved in the giant cell formation which in turn may undergo autolysis.

12. Intranuclear inclusions of monocytes may be found free in the tissue spaces and there phagocytosed by macrophages. This is taken as evidence that they are more resistant than the cells producing them.

13. Intranuclear inclusions develop in the duct cells of the

submaxillary gland as early as the 6th day after inoculation, and complete their development to the old stage 21 to 24 days after inoculation, which is 15 to 19 days after they first appear. Cytoplasmic inclusions develop about the middle of the cycle.

14. The early stages of nuclear reactions in the submaxillary gland duct cells include those changes that were called "early" and "intermediate" in the monocytes. The intermediate stages in duct cells involve migration of some of the granules of chromatin to the inclusion body, but these are scattered over the inclusion and are not clumped. The late stages are those in which most of the chromatin has migrated to the inclusion body and has aggregated into clumps.

15. The reaction of nuclei to inclusions of virus origin have been compared to the chemically produced inclusions of Olitsky and Harford and it is suggested that the differences are due to those of intensity of the reaction on the nucleus rather than differences in quality.

16. Several lines of evidence indicate that cells containing intranuclear inclusions are living and not dead.

17. Centrifugation of inclusion-bearing submaxillary duct cells at young and intermediate stages reveals that the chromatin is the heaviest element in the nucleus, that the inclusion is next, and that the nuclear sap is lightest, which results are contrary to those found for herpes. The cytoplasmic inclusions are as heavy as the nucleus.

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## DESCRIPTION OF PLATES

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Drawings were made with a camera lucida at a magnification of  $\times 3250$  and are reduced to a magnification of  $\times 1800$ . The direction of centrifugal force in Figs. 12, 13, 14, 26, 27 and 28 is indicated by an arrow beside each figure.

AIN = acidophilic inclusion; ABIN = acidobasophilic inclusion; CIN = cytoplasmic inclusion; ININ = intranuclear inclusion in cytosome of a macrophage; LUMEN = lumen of a submaxillary gland duct; and NL = nucleolus.

The cells shown in Figs. 1-15 were stained with Ehrlich's hematoxylin and eosin stain, and all except those in Figs. 9 and 10 were fixed in Zenker's fluid plus acetic acid. In these 2 cases the fixative was an alcohol and corrosive sublimate mixture.

### PLATE 55

FIG. 1. An early stage in the formation of an intranuclear inclusion. A monocyte from the meninges of the brain of an adult guinea pig, which died 5 days after an intracerebral inoculation of 20 per cent submaxillary gland virus.

FIG. 2. A monocyte with two moderately large intranuclear inclusions. The nuclear reaction to the inclusion is at about the same stage as in Fig. 1. From the same slide as Fig. 1.

FIG. 3. An early stage in the nuclear reaction to an inclusion formation but later than that illustrated in Figs. 1 and 2. A monocyte from the meninges of the brain of an adult guinea pig, 1 of 3 animals in Group III, Table II, which died 8 days after intracerebral inoculation.

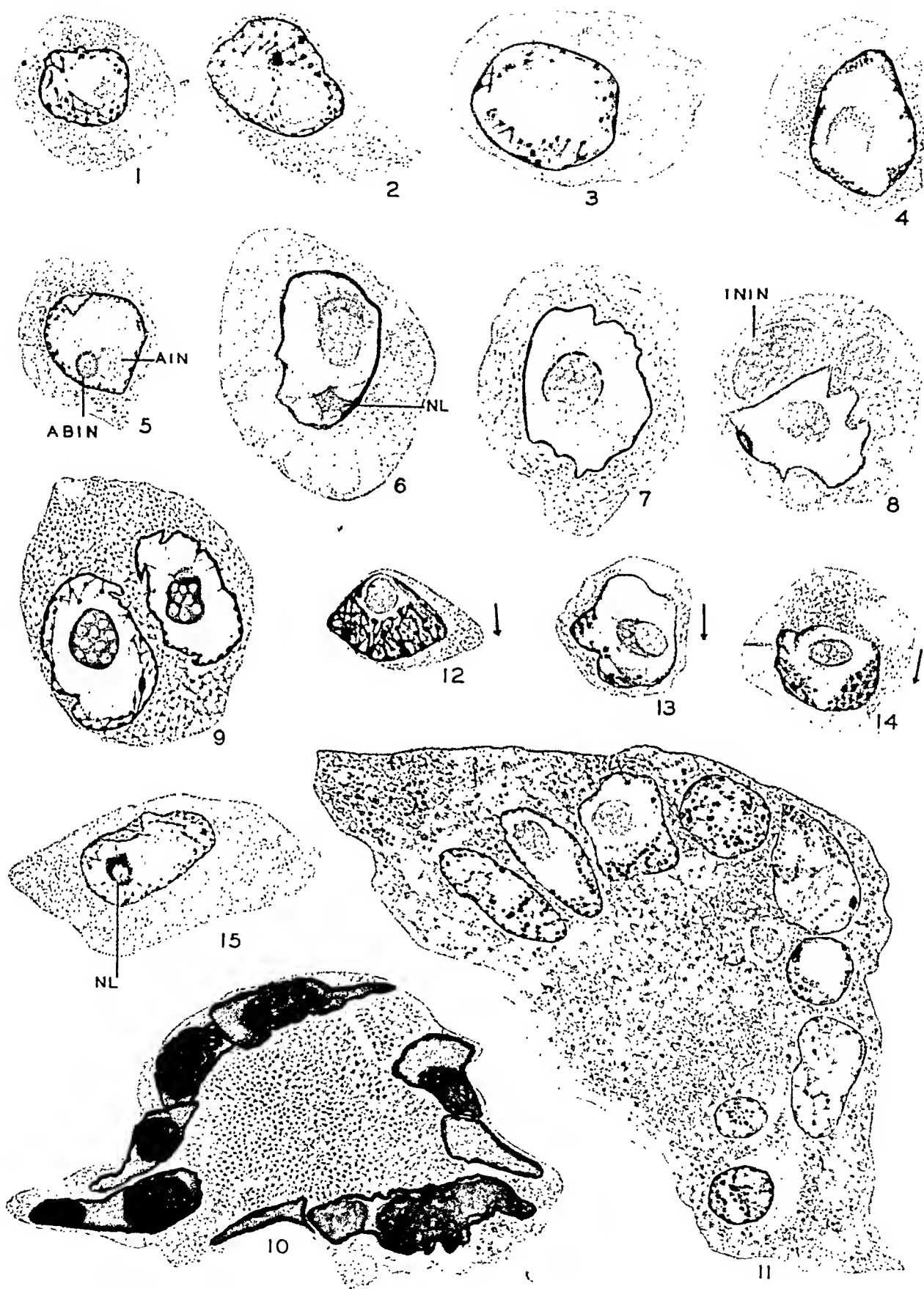
FIG. 4. An intermediate stage in the nuclear reaction to an inclusion formation. A monocyte from the meninges of the brain of an adult guinea pig, Group II, Table II, which was sacrificed at coma stage  $4\frac{1}{2}$  days after intracerebral inoculation. The acidobasophilic body forms a cap at one side of the acidophilic body.

FIG. 5. About the same stage of nuclear reaction as shown in Fig. 4. Taken from the same slide. The acidophilic and acidobasophilic bodies are separate, a condition that may be found occasionally.

FIG. 6. A late stage in nuclear reaction. Most of the chromatin, except the nucleolus, is completely margined. Acidophilic and acidobasophilic inclusions are shown. A large monocyte taken from the same slide as that shown in Fig. 3.

FIG. 7. The latest stage of nuclear reaction observed until autolysis sets in. The margined chromatin shows a slight tendency to aggregate into regular clumps along the margin. A small amount of chromatin has returned to the surface of the inclusion body. A large monocyte from the same slide as that shown in Figs. 4 and 5.

- FIG. 8. A large monocyte which has phagocytozed three intranuclear inclusions liberated from other cells. Its own nucleus is in a late stage of nuclear reaction. A cell from the meninges of the brain of adult guinea pig No. 129, Table III, which died 7 days after intracerebral inoculation.
- FIG. 9. A binucleated large monocyte. The nucleus to the left shows an early stage of reaction and the one to the right an intermediate stage. From the meninges of a 5 weeks old guinea pig (No. 149) sacrificed at coma stage 5 days after intracerebral inoculation.
- FIG. 10. A small epithelioid giant cell undergoing autolysis. The inclusions are nearly obscured by the liquefied chromatin. From the same slide as Fig. 9.
- FIG. 11. An epithelioid giant cell showing varying stages of nuclear reaction to inclusion bodies. Some nuclei are undergoing autolysis or else have been phagocytozed. From the meninges of a guinea pig, Group I, Table II, which died 4 days after intracerebral inoculation.
- FIG. 12. A lymphocyte with an intranuclear inclusion which has been centrifuged for 30 minutes at forces between 300,000 to 550,000 times gravity. Oxychromatin and basichromatin moved to the centrifugal pole and the inclusion body is pushed to the opposite pole. From the meninges of a guinea pig, Group I, Table II, which died 4 days after intracerebral injection.
- FIG. 13. A monocyte which has been centrifuged long enough to pull much of the chromatin toward the centrifugal pole but which does not show much displacement of the inclusion body. From the same slide as Fig. 12.
- FIG. 14. A monocyte which contains a greater quantity of chromatin than Fig. 13. The inclusion body is displaced slightly toward the centripetal pole. From the same slide as Fig. 12.
- FIG. 15. Inclusion-bearing cell from the liver. The karyosome and plasmosome nucleoli are toward the left and the acidophilic inclusion to the right of the center. A 5 weeks old guinea pig, No. 140, Table IV, which had been injected with the virus into the spleen 7 days before death.





# THE HISTOLOGY OF ORBITAL AND OTHER FAT TISSUE DEPOSITS IN ANIMALS WITH EXPERIMENTALLY PRODUCED EXOPHTHALMOS \*

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In the many attempts to produce exophthalmos experimentally, stimulation of the sympathetic nerves which causes a contraction of the smooth muscles of the orbit has almost invariably been used.<sup>1-5</sup> None of these methods has produced an exophthalmos comparable to the more marked clinical cases, although some degree of proptosis has been obtained. Recently examination of the orbital contents of animals with exophthalmos produced by injection of extract of the anterior lobe of the pituitary gland revealed many features characteristic of clinical exophthalmos following thyroidectomy (Smelser<sup>6</sup>). The weight of the orbital contents, muscles, lacrimal glands and fat tissue deposits was increased in these animals and the fat tissue, and to some extent the muscles, became edematous. The edema, associated with some round cell infiltration of the orbital fat tissue and muscles, presented essentially the same condition as that found in orbital tissues in exophthalmos following thyroidectomy. The proptosis produced was marked (not simply a widened palpebral fissure) and persisted postmortem. The protrusion could be convincingly demonstrated and measured by a roentgenogram of the orbit. The position of the bulb was indicated by a silver marker placed on the apex of the cornea.

These observations assume especial importance because of clinical studies of exophthalmos which have been made in recent years.<sup>7-10</sup> All of these studies have been concerned with malignant or progressive exophthalmos which increased following thyroidectomy. Similarly, the exophthalmos produced by injection of extract of the anterior lobe of the pituitary gland is most severe when the metabolic rate is low (Friedgood,<sup>11</sup> Smelser<sup>12</sup>), or in the absence of the thyroid (Marine and Rosen,<sup>13</sup> and Smelser<sup>6, 14</sup>). Examination of orbital contents from patients with exophthalmos

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has revealed in many instances enlarged extraocular muscles which are found on histological examination to be edematous and invaded by masses of round cells. Other cases are reported in which edema and hypertrophy of the orbital fat and connective tissue are considered to be the cause of the exophthalmos.<sup>15-17</sup> Further evidence indicates that the edema is general throughout the orbit, affecting both the fat tissue and the muscles.<sup>6</sup> Study of biopsy specimens from different regions or muscles of an orbit does not, however, show them to be necessarily equally affected, thus suggesting an explanation of the various changes observed in such cases.

The production of exophthalmos and edema of fat and connective tissue of the orbit by injection of extract of the anterior lobe of the pituitary gland raises the question whether this reaction, in widely distributed tissues, is localized in the orbit or occurs generally in the fat tissue deposits.

### EXPERIMENTAL

Nineteen full grown guinea pigs were thyroidectomized, and following a 15 to 20 day recovery period were injected with an extract of anterior lobe of the pituitary glands of beef. The extract was prepared as described earlier<sup>18</sup> and was essentially a well tolerated, partially purified preparation which produced body growth of hypophysectomized rats, greatly increased weight of the thyroids and ovaries in guinea pigs and of adrenals of thyroidectomized guinea pigs. An amount of extract equivalent to 250 to 500 mg. of acetone-dried powdered anterior lobe of the pituitary gland was injected daily for various periods of time. Exophthalmos became evident in most cases within 15 to 20 days, although in a few instances 30 to 40 injections were necessary. An autopsy was performed after exophthalmos had been maintained approximately 20 days. The orbital contents were removed, weighed, and the fat tissue fixed in Bouin's fluid. All animals were exsanguinated before the tissues were removed. Fat tissue from the cervical region lying lateral to the trachea and extending posteriorly from the larynx, was also fixed. Other samples of fat tissue were taken from the axilla and the kidney. The fat tissue attached to the ovary, fallopian tube and upper portion of the uterus was taken from the females, and a specimen

surrounding the ureter near the bladder from the males. By this means fat deposits in several regions were examined and compared with orbital fat tissue from the same animal. The specimens were all fixed in Bouin's fluid, embedded in paraffin and sections cut at  $6\mu$  and stained with Masson's trichrome method and the hematoxylin-eosin stain. Sections from several regions in each specimen were used in order to study representative areas. Control tissues were obtained from 6 normal and 4 thyroidectomized uninjected guinea pigs. Nine thyroidectomized animals, 4 of which received extract of liver and 5 of which received thymus extract, provided additional tissues for comparison. The beef liver and thymus extracts were prepared by the same method used for the pituitary glands and were injected in daily amounts equivalent to 1000 mg. of the acetone-dried tissue.

## RESULTS

Sixteen of the animals injected with pituitary extract became definitely exophthalmic and were autopsied. The orbital contents were carefully examined and weighed, and were fixed for histological study. Injection of pituitary extract produced a 35 per cent increase in total weight of the retrobulbar tissues. All orbital structures were involved in this increase with the exception of the ventral lacrimal gland. The orbital fat tissue was proportionally the most affected, although the dorsal lacrimal gland, because of its size, contributed more to the total weight of the orbital contents. Swelling of the extraocular muscles was of particular interest because of the emphasis placed on them in clinical studies and because their weight was constant in the control animals. These observations have been discussed more completely elsewhere.<sup>6</sup> Injections of liver and thymus extracts were without effect on the weight of the orbital contents of the control animals.

The structural modifications of the dorsal lacrimal and extraocular muscles will be discussed separately. The amount of orbital fat tissue in the exophthalmic animals was not only increased but its appearance was definitely changed. Normally, and in the injected control animals, orbital fat tissue was rather opaque, but in the exophthalmic animals it appeared to be translucent and gelatinous. The amount of orbital fat tissue was nearly double that of the control animals, weighing an average of 63 mg. in the

animals injected with pituitary extract and 27 to 33 mg. in the control animals. Orbital fat tissue from normal and uninjected thyroidectomized guinea pigs weighed 31 and 33 mg., and that from control animals treated with liver and thymus extract weighed 27 and 33 mg. respectively.

*Orbital Fat Tissue:* The orbit of the normal guinea pig contains a small amount of fat tissue which lies directly behind the bulb, surrounding the optic nerve and filling the interstices between the extraocular muscles and, to some extent, between the lobules of the large dorsal lacrimal gland. The bulk of the fat tissue lies within the muscle cone which it fills. It is traversed by nerves, small blood vessels and the striated retractor-bulbi muscles, which are very small. The entire orbital fat content was studied and sections revealed many fat cells divided by connective tissue septums into irregular lobules. Because all autopsies were performed after the animals had been exsanguinated, no extravasation occurred to confuse the study. The collagenous connective tissue septums contained in all instances a variable but small amount of fluid. This material which stained faintly with acid dyes was found in the loose connective tissue of other fat deposits and in the orbital fat tissue of other species, including man. Injection of large amounts of extract of liver and thymus gland did not affect the orbital fat tissue of 6 of the 9 treated animals. One animal seemed to have slightly more edematous material in the connective tissue than is seen normally, and 2 animals as much as in the animals in the series treated with pituitary extract.

The orbital fat tissue of the guinea pigs treated with extract of the anterior lobe of the pituitary gland differed markedly from that of the control animals (Figs. 1 and 2). The fat cells which normally constitute the major portion of each section appeared reduced in the animals injected with pituitary extract because of an edematous infiltration which seemed in most cases to occupy nearly the entire section. The connective tissue fibers appeared to be divided and dispersed in the infiltration. The edematous fluid also penetrated between fat cells, although not in as marked a degree as between the connective tissue fibers. In many instances, however, instead of a solid mass of fat cells the edematous infiltration seemed to have scattered them so that they appeared to be floating freely in the infiltrating medium.



The edematous material stained deeply with aniline blue or eosin and did not give any staining reactions characteristic of amyloid or mucoid infiltration. Although the general appearance under low magnification was homogeneous, observation of the infiltrate with higher magnification revealed many minute acidophilic granules. Orbital fat and connective tissue, and extraocular muscles from several individuals who developed exophthalmos following thyroidectomy were found to be infiltrated with a material similar in all respects to that produced experimentally in similar tissues in the guinea pig.

The relative amounts of this infiltrating material found, expressed in arbitrary units, are shown in Table I. The valuations assigned were determined without reference to serial number of the animal and without knowledge of the treatment which had been given.

*Axillary Fat Tissue:* Specimens were removed from the axilla at autopsy. Nearly the entire mass of fat tissue was taken so that the sections are representative of the whole. Tissue from this region in the control animals consisted of solid masses of fat cells irregularly divided by connective tissue septums. As in the orbital fat tissue, blood vessels and nerves were found in the sections, although the axillary fat tissue was less vascular than that found in the orbit, and much less connective tissue was present. Only 7 of the 19 control animals showed small amounts of edematous material similar to that noted in the normal orbital connective tissue. Injections of thymus and liver extracts were apparently without effect on the axillary fat tissue in all instances.

Fat tissue taken from the axillary region of exophthalmic guinea pigs treated with pituitary extract did not differ from that of the control animals. About one-third of the preparations revealed a small amount of edematous material in the connective tissue which in no instance was greater than that in the animals treated with extract of liver and thymus or in those that were uninjected.

*Cervical Fat Tissue:* The specimens of cervical fat tissue were taken from the region lateral to the trachea, near the site of the thyroid gland, which had been removed at the beginning of the experiment. Normally, fat tissue from this region is intermediate between the axillary and orbital fat tissue in general histological

TABLE I

*Relative Amount of Edematous Infiltration Found in Various Parts of the Body*

Animal number	Sex	Orbital fat tissue	Axillary fat tissue	Cervical fat tissue	Renal fat tissue	Ovarian or ureteral fat tissue
<i>Normal Uninjected Guinea Pigs</i>						
646	♀	+	+	+	o	o
242	♀	+	o	+++	o	o
245	♀	++	+++	++	+	o
246	♀	++	o	o	o	
247	♂	+	+++		o	
238	♂	+	++	+++	o	+
<i>Uninjected Thyroidectomized Guinea Pigs</i>						
303	♀	++	o	+	o	+
304	♂	++	o	+	o	o
305	♂	++	o	++	o	
454	♀	++	o	o	o	o
<i>Thyroidectomized Guinea Pigs Injected with Liver Extract</i>						
271	C.F.	++++	o	+++	o	
275	C.M.	+	o	o		
301	♀	+	o	o	o	o
302	♀	++	+	o	o	o
<i>Thyroidectomized Guinea Pigs Injected with Thymus Extract</i>						
267	C.F.	++	o	+++	o	
268	♂	+++	o	o	o	
270	C.M.	++++	o	o	o	
299	♀	++	++	+++	o	
300	♀	+	+	+++	o	
<i>Thyroidectomized Guinea Pigs Treated with Extract of the Anterior Lobe of the Pituitary Gland</i>						
199	♀	++++	+	+++	+	++
219	♀	++++	+	+++	o	+++
225	♀	++++	o	+	o	o
241	♀	++	o	o	o	o
261	♀	++++	++	+	o	o
262	C.F.	++++	o	o	+	
263	C.F.	+++	o	o	o	
265	♀	++	o	+	o	o
273	♀	++++	+	+	o	+++
285	♀	++		+		
287	♀	++++	++	+++	++	++++
288	♀	++++	o	++	o	o
289	♀	+++		++		
290	♀	++		+		
291	♀	++++	+	+	o	+++
292	♀	++++	o	o		
294	♀	++++	o	++	++	++++
295	♂	++++	o	++	o	++
297	♂	++++	++	++	o	+++

o = A minute trace or no infiltration.      + = A little infiltration observed.  
 ++ and +++ = Intermediate degrees of infiltration.

++++ = Extreme infiltration, the entire section being affected.

All valuations were assigned without reference to serial numbers or treatment.  
 C.F. = Castrated Female; C.M. = Castrated Male.

appearance. There is more connective tissue and vascularity present than is seen in the specimens from the axillary region but less than in those from the orbital area. The edematous material noted in the connective tissue of the axillary and orbital fat tissue was found in this series in 11 of the 18 guinea pigs studied. The amount was greater than that found in the axillary region and nearly as much as that found in the orbit. Injection of thymus and liver extracts did not affect its distribution or amount.

Injection of extract of the anterior lobe of the pituitary had apparently little or no effect on the cervical fat tissue. Fifteen out of 19 animals exhibited a small amount of edema in the connective tissue bands, a slightly higher proportion than in the control animals, but in none of them was it more extensive than is seen normally (Figs. 3 and 4).

*Renal Fat Tissue:* The mass of fat tissue partly surrounding and covering the peritoneal side of the kidney was the fourth fat deposit studied. Structurally it differed considerably from all the others. No nerves and very few blood vessels passed through it and the amount of connective tissue present was the least in all the specimens studied. Sections from the normal control animals revealed a solid mass of fat cells which were not divided into irregular lobules by connective tissue septums, as were those from the cervical and orbital regions. In only one instance did the normal and thyroidectomized control animals reveal any of the edematous material characteristic of normal tissues from the orbit, neck and axilla. Injections of thymus and liver extract did not modify this condition in any instance.

The renal fat tissue from the exophthalmic animals treated with pituitary extract, however, was slightly affected. One-fourth of the specimens showed some accumulation of edematous infiltration in the connective tissue. In one instance this was sufficient to penetrate and partially disperse some of the fat cells. In no instance, however, was the condition extreme, but the deviation in these animals from that seen in the highly consistent control animals was convincing.

*Ovarian Fat Tissue:* The sections containing ovarian fat tissue differed from the other preparations because of the several different types of tissue present (Figs. 5 and 6). Each section passed through the ovary, fallopian tube and the uterus, and the mesen-

tery and fat tissue attached to them. The fat tissue was divided into lobules by septums and penetrated the connective tissue support of the fallopian tubes. Many smooth muscle fibers were present in this region. The edematous material noted in other normal fat tissue was present, in a very small amount, in only one specimen, and the ovarian fat tissue from the animals treated with liver extract revealed none of this infiltration. Fat tissue from this region of the guinea pigs treated with thymus extract was not studied.

Ovarian fat tissue from 10 animals treated with pituitary extract was studied. Six of these specimens contained considerable amounts of edematous material, all of them more than that in any control animal, and four contained large amounts of edematous material. The condition of 2 animals was judged as extreme as evidenced by the appearance of the orbital fat tissue.

*Ureteral Fat Tissue:* Very few specimens of fat tissue from the ureters of male animals were examined. Normally they consisted of irregular lobules of fat tissue separated by septums of connective tissue fibers. Some nerves and a few blood vessels penetrated this mass. The connective tissue from 1 of the uninjected animals contained a small amount of edema, such as was noted in other control animals, and a second specimen contained none (Fig. 7). No material was studied from animals injected with liver or thymus extract.

Two specimens of fat tissue were taken from the ureters of male animals treated with extract of the anterior pituitary gland. Both specimens showed considerable edematous infiltration of the connective tissue. This edematous material penetrated through the connective tissue septums and to lesser degree throughout the fat cells, tending to isolate them (Fig. 8). The infiltrating material, although much less in amount than that in the orbit, possessed the same staining characteristics and texture as the orbital and the other fat tissues.

## DISCUSSION

The data shown in Table I demonstrate that the amount of edematous material found in the fat tissue studied not only varies widely in amount between the exophthalmic and the control series of animals, but also between the various fat deposits in the same

animals. If averages of the ratings for the control animals and those treated with pituitary extract are studied, this is very apparent (Table II). Normally the orbital fat tissue contains more edematous material than any of the other tissues, axillary fat tissue very little, and renal fat tissue none at all. In the exophthalmic animals, or those injected with pituitary extract, this re-

TABLE II

*Average Degree of Edematous Infiltration of the Fat Deposits in Control and Exophthalmic Animals*

	Orbital fat tissue	Axillary fat tissue	Cervical fat tissue	Renal fat tissue	Ovarian fat tissue	Ureteral fat tissue
Controls	1.9	0.6	1.38	0.05	0.1	0.5
Exophthalmic	3.5	0.6	1.36	0.4	1.9	2.5

The figures are the average of + signs in their respective groups. Ureteral fat tissue values represent only 4 cases.

lationship is preserved, the orbital fat tissue containing much more edematous infiltration.

Injection of pituitary extract increases not only the degree of edema but also the percentage of instances in which it is found (Table III). It may be worthy of note that, with the exception of the orbital, only fat tissue in the peritoneal cavity was much affected by the injection of pituitary extract.

TABLE III

*Percentage of Specimens in which any Trace of Edematous Infiltration Was Found*

	Orbital fat tissue	Axillary fat tissue	Cervical fat tissue	Renal fat tissue	Ovarian fat tissue	Ureteral fat tissue *
	%	%	%	%	%	%
Controls	100	36.8	61.1	0.05	14	50
Exophthalmic	100	43.7	79	26.6	60	100

\* The figures on ureteral fat tissue are based on only 2 cases in each series of animals.

The difference between the various fat tissues in their reaction to pituitary extract may be, in part, explained on a basis of structure. Orbital fat tissue, which exhibits the most extreme degree of edema in exophthalmos and which even shows traces of a similar material normally, contains many septums of loose connective tissue. It is in these bands of collagenous fibers that the edematous infiltration is first found and is most extensive. Renal fat tissue, which contains practically no connective tissue, is the least affected.

This structural feature may explain why endocrine products circulating generally seem to have a rather specific effect (edema of the orbital fat and connective tissue) on a widely distributed tissue. The present study indicates that the same process as that taking place in the orbit is occurring generally in peritoneal fat deposits. It is more extreme in the orbital region because of the structure of its fat tissue. Any swelling of the orbital fat tissue, particularly in man, where it fills most of the retrobulbar space, is immediately noticeable because of its effect on the protrusion of the eye, whereas a slighter degree of edema elsewhere would pass unnoticed. The degree of edema produced in fat tissue from the peritoneal cavity was too slight, relative to the weight of the entire mass, to affect the weight or appearance except microscopically.

By histological study one can determine fairly accurately the location of the edematous infiltration in question but accurate quantitative data on the amount of water, fat and connective tissue cannot be ascertained. This can be done only by chemical analysis of the tissues in question. Such a study in its preliminary stages confirms the general observations reported here.

### CONCLUSIONS

1. The injection of extract of the anterior lobe of the pituitary gland produces exophthalmos and marked edema of the orbital fat and connective tissue.
2. Fat tissues in various parts of the body are also affected by the injections of anterior pituitary extract but to a lesser degree than that in the orbit.
3. The difference in degree of edema produced in the orbit and that found in other fat tissue deposits may be due to the difference in structure of the orbital fat tissue.
4. The edematous material found in the orbit appears to be identical in composition with that found elsewhere in the same animal.

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## DESCRIPTION OF PLATES

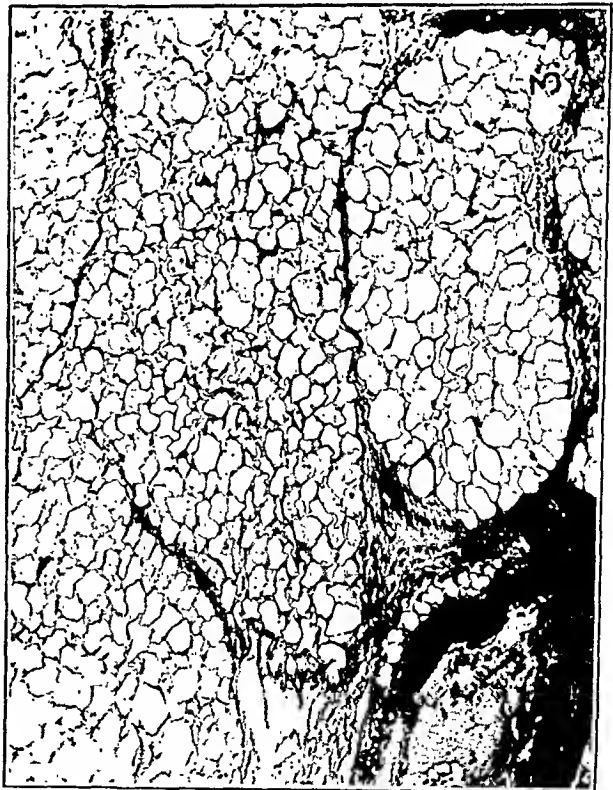
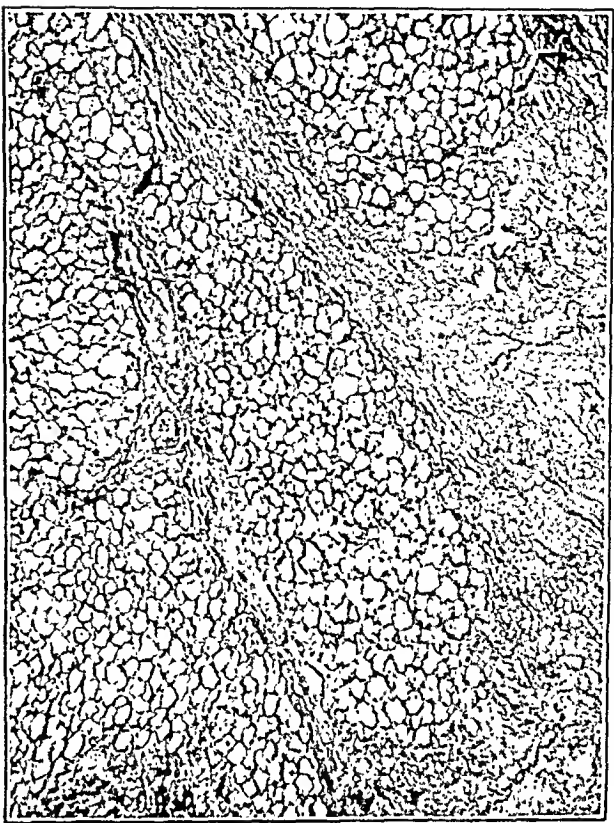
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### PLATE 57

All microphotographs were taken at a magnification of  $\times 45$ .

1. Orbital fat tissue from a guinea pig (No. 275) injected with liver extract.
2. Orbital fat tissue from an exophthalmic guinea pig (No. 261) injected with pituitary extract. Note the marked edematous infiltration which disperses the connective tissue fibers and almost entirely replaces the fat cells.
3. Cervical fat tissue from an untreated thyroidectomized guinea pig (No. 305).
4. Cervical fat tissue from an exophthalmic guinea pig (No. 287). This particular specimen shows slightly more edematous infiltration than the average.



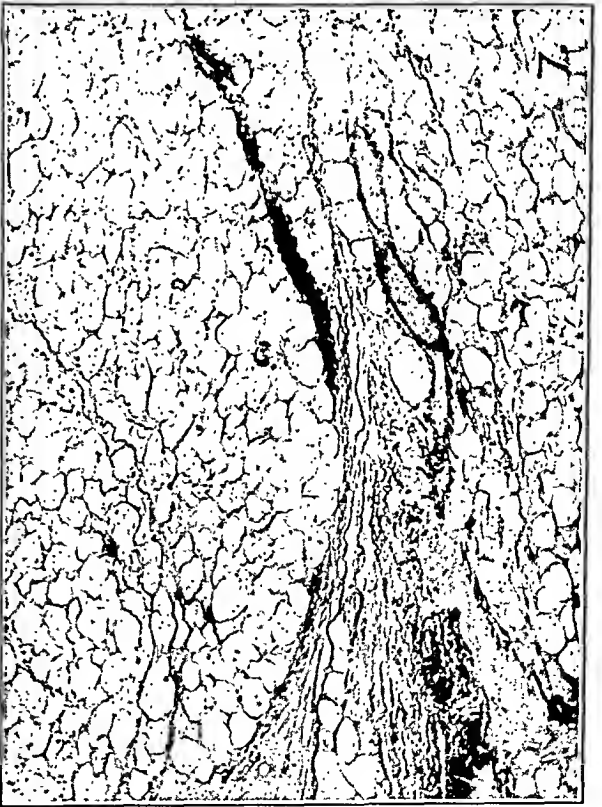
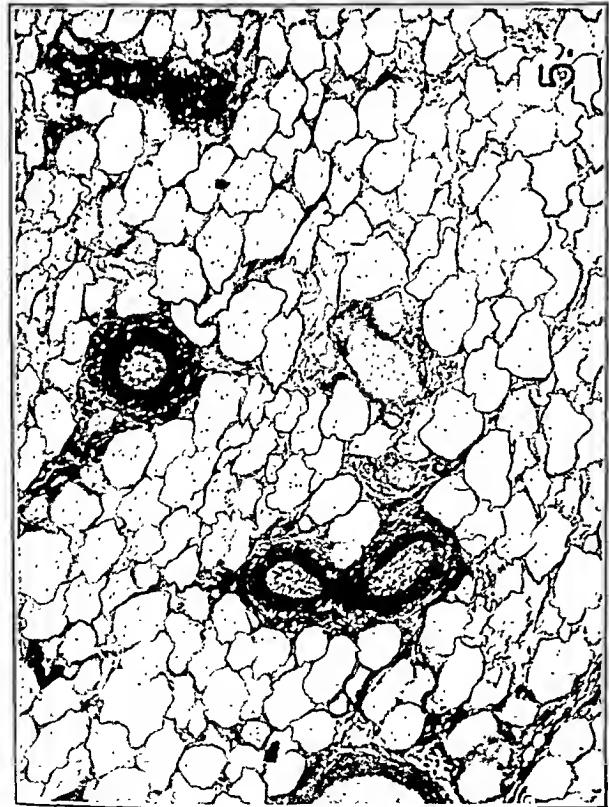
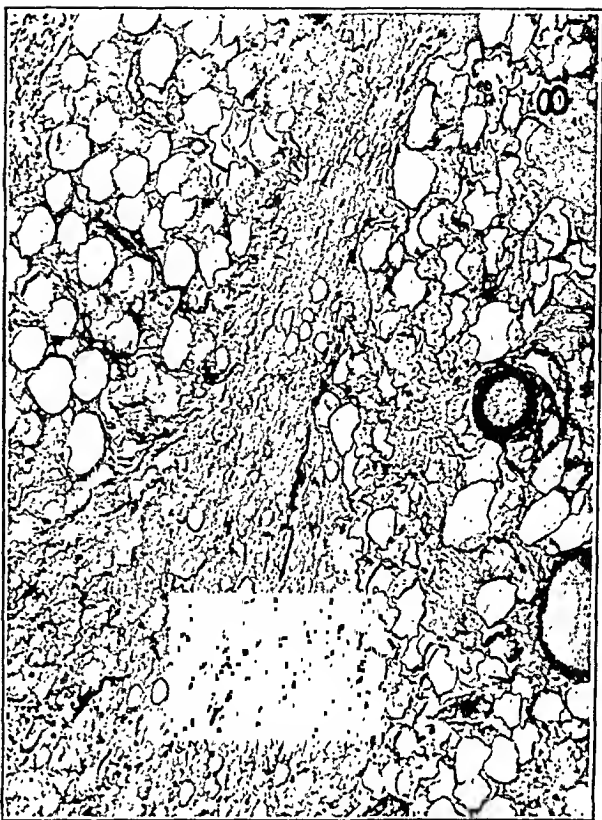


Smelser

Fat Tissue in Experimental Exophthalmos

PLATE 58

5. Ovarian fat tissue from an untreated thyroidectomized guinea pig (No. 303).
6. Ovarian fat tissue from an exophthalmic guinea pig (No. 294). Note the marked edema in the connective tissue and between the fat cells. Sections of the fallopian tubes are seen.
7. Fat tissue from the ureter of a normal male guinea pig (No. 238).
8. Fat tissue from the ureter of an exophthalmic male guinea pig (No. 297) injected with pituitary extract. Compare with Figs. 2 and 6.





## CEREBRAL VASCULAR DISEASE ACCOMPANYING SICKLE CELL ANEMIA \*

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Patients with sickle cell anemia accompanied by cerebral vascular accidents have been observed by several authors.

Cook <sup>1</sup> first reported a case of a negro boy aged 7 years who expired following sudden loss of consciousness. The autopsy showed subarachnoid hemorrhage associated with cerebral softening.

Arena <sup>2</sup> reported the case of a negro boy aged 6 years who had headache, vertigo, stupor and right hemiplegia. A footnote to this article cited a similar case observed in a negress aged 4 years. Arena mentioned personal communications from Sydenstricker and Cooley citing 3 other cases and suggested thrombosis of cerebral vessels as a possible cause of the neurological changes.

Yater and Hansmann <sup>3</sup> reported a case of a negress aged 38 years who had neurological manifestations. An autopsy showed recent diapedesis of red cells into the meninges over the right hemisphere, a small area of softening 1 cm. in diameter in the cortex of the left parietal region, and a uniformly shrunken, sclerosed choroid plexus with calcium encrustations.

Ford <sup>4</sup> mentioned several negro children with dural sinus thrombosis who had had sickle cell anemia.

A case of a negro boy, aged 10 years, who had repeated symptoms referable to the central nervous system with residual spasticity on the right side, was reported by Kampmeier.<sup>5</sup>

Harden <sup>6</sup> described vascular changes in the retina and extremely tortuous and thickened superficial temporal vessels in 2 negro children with sickle cell anemia.

Arena <sup>7</sup> has also observed 4 negro boys, aged 4, 5, 6 and 10 years, who showed neurological symptoms and signs and who had sickle cell anemia.

In none of the cases thus far reported has an adequate description of the actual condition of the cerebral vessels been recorded,

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and no satisfactory account of the origin and nature of the cerebral clinical disturbances has been offered.

The following account of the clinical and pathological study of 2 cases of sickle cell anemia accompanied by cerebral vascular manifestations is recorded because of its contribution to the pathogenesis of the disorders of the central nervous system in sickle cell anemia and to the general problem of the pathology of the disease.

### CASE REPORTS

**CASE 1. Clinical History:** A negress, aged 4 years, first entered the Duke Hospital on Feb. 8, 1934, with a history of paralysis of 3 weeks duration. At the onset of the attack the child suddenly became speechless and saliva drooled from her mouth, which drooped. Two weeks later the child lost the use of her right arm and leg. The family history was interesting in that 3 other siblings died with a similar condition. In 1 of these, sickle cell anemia had been diagnosed in our clinic. This sibling died at home after having been followed in the Duke Hospital Clinic.

**Physical Examination:** The temperature was 37.5° C., pulse 100, respirations 26, and the blood pressure 105/65 mm. Hg. The child could neither walk nor speak, but could comprehend commands and questions. The tongue could not be protruded or moved from side to side. The spleen was palpable 2 to 3 fingerbreadths below the costal margin. Paralysis, weakness, and spasticity of the right arm and leg were present. The reflexes were hyperactive throughout but more marked on the right side. The Babinski, Kernig and Brudzinski signs were all absent. A lumbar puncture showed no abnormality of the spinal fluid.

**Accessory Clinical Findings:** A fresh blood smear showed 100 per cent sickling in 4 hours; the hemoglobin was 52 per cent (7.8 gm.) Sahli, and the white blood cells numbered 24,000. The indirect van den Bergh test was slightly positive (trace). The patient was discharged from the hospital with a diagnosis of sickle cell anemia with cerebral thrombosis in the region of the left internal capsule.

In an interval of 3½ years, during which time the patient was followed in the Duke Pediatric Clinic, the use of the right arm and leg returned and speech returned almost to normal. The finer movements with the right hand, however, such as writing, buttoning clothes or holding table silver, could not be performed. A residual limp and dragging of the right leg persisted.

The patient was again admitted to the hospital 3½ years later. Fifteen hours previous to the second admission she suddenly covered the frontal region of her head with her left hand and cried out that her head hurt. She immediately crumpled to the floor, respirations became labored, and both legs became paralyzed. Inability to speak followed.

**Physical Examination:** The temperature was 39.0° C., pulse 120, respirations 38, and the blood pressure 150/100 mm. Hg. The child, now 7½ years of age, was small and undernourished and lay unconscious in bed with mouth and eyes open, breathing rapidly and irregularly. The lungs were clear. A soft, blowing systolic murmur was heard over the entire precordium but best over the mitral area. The eyes moved aimlessly at times but tended toward

left horizontal deviation. The spleen was not palpable. Both arms were spastic, the right more so than the left. The left leg was flaccid. Supraorbital pressure caused purposeful movements with both arms, in an attempt to knock away the examiner's hands, and the left leg became spastic. The tendon reflexes were hyperactive on both sides, more marked on the right. The abdominal reflexes were absent. The Kernig and the Babinski tests were positive bilaterally. A response to painful stimuli was present over the entire body.

*Accessory Clinical Findings:* The hemoglobin was 40 per cent (6.7 gm.) Sahli, red blood cells 1,500,000, white blood cells 50,000, with polymorphonuclear leukocytes 94 per cent. Sickling was 25 per cent, immediate; 95 per cent in 24 hours. The urine contained albumin (4+) and 5 to 10 granular and cellular casts per high power field. The spinal fluid showed an initial pressure of 270 mm., was bloody, with a slight xanthochromia and contained 30,000 red blood cells. The Wassermann and colloidal mastic tests were negative.

The patient was restrained, placed in a dark room, and was given sedation with an infusion of 2.5 per cent glucose. At this time the temperature was 105.8° F. No change followed and she expired quietly 15 hours later.

### *Autopsy Report*

*Macroscopic Examination:* The heart was slightly dilated but not hypertrophied. The spleen (Fig. 5a and b) weighed only 1.5 gm. and measured 3.2 by 1.5 by 1 cm. It was triangular, covered with many adhesions, firm, wrinkled and grayish pink with a few small bluish areas scattered over the surface. The cut surface showed a thick capsule with prominent trabeculae and gray pulp with a few brown spots throughout. In one area a large reddish brown hemorrhage 1 cm. in diameter was present.

When the dura was opened a large amount of fluid blood was evacuated. Both subdural and subarachnoid hemorrhages in the form of blood clots covered the brain (Fig. 8), the clots being more marked over the right optic tract, anterior portion of the cerebellum, right Sylvian fissure and right insula. An area of encephalomalacia involved the left Rolandic fissure, the left posterior end of the Sylvian fissure, the left superior temporal gyrus and the entire left frontal lobe. The gyri of the left frontal and occipital lobes appeared small in contrast with those of the right except for the right middle frontal convolutions which also appeared atrophic. The right middle cerebral artery was buried in a large fresh blood clot between the right frontal and right temporal lobes. A blood clot measuring 4 cm. in diameter (Fig. 9) was found in the region of the right insula at the level of the anterior commissure.

Sections of the medulla showed a marked difference in the size of the right and left pyramidal tracts (Fig. 6), the right tract being much smaller than the left. The left lateral ventricle was greatly dilated (Fig. 9), being two to three times the size of the right.

The diameter of the middle cerebral arteries was smaller where they branched from the internal carotids, at the circle of Willis, than in the distal portions.

*Microscopic Examination:* Many sickled erythrocytes were seen in a preparation of heart's blood in normal saline at the time of autopsy.

The splenic structures were distorted, with a predominance of connective tissue, especially at the periphery where the capsule was wrinkled and provided with many hyaline infoldings (Fig. 1). The cells of the pulp consisted mainly of irregularly shaped lymphocytes, plasma cells and sickle shaped erythrocytes. Throughout the connective tissue there could be seen large deposits of iron and calcium, some being in the form of rings (Fig. 1), the latter under oil immersion appearing irregular and fragmented. The splenic vessels (Fig. 2) showed intimal proliferation, disrupted internal elastic lamina with iron and calcium deposits, fragmented muscular coats with pyknotic nuclei, hyaline walls with necrotic areas, and a few perivascular hemorrhages. One large hemorrhage contained pale staining sickle shaped erythrocytes, fibroblasts and minute deposits of iron pigment. The lumens of many vessels were nearly obliterated by periarterial fibrosis and intimal proliferation. Typical malpighian corpuscles were not noted.

The cerebral vessels showed endarteritis and, in several instances (Figs. 3 and 4), obliteration of the lumen with endothelial proliferation, fibroblastic reaction and hyalinization, occasional loss of internal elastic lamina, and fragmentation of the persistent internal elastic lamina with iron deposits. A few inflammatory cells were seen in the intima, media and adventitia. Perivascular hemorrhages and necrosis of the muscle layers with pyknosis and fragmentation were also present. No cholesterol crystals were noted and the elastic lamina showed thickening but no splitting, as described by Tuthill.<sup>8</sup> The lumens of the right middle cerebral artery and left ascending branch of the left middle cerebral artery were completely obliterated; other vessels with partial obliteration



tion were the left middle cerebral, and right and left anterior cerebral arteries. The left internal carotid artery showed only breaks in the internal elastic lamina but no intimal thickening.

The brain contained many areas of focal necrosis usually associated with perivascular hemorrhages. Almost complete degeneration was found throughout the left frontal cortex and left pyramidal tract above the decussation. No proliferation of endothelium was noted in the minute vessels of the brain substance. The large blood clot in the region of the right insula was carefully dissected for possible aneurysm but none was found. Serial sections of the small vessels showed only complete breaks in the walls without inflammatory reaction or necrosis.

No abnormality of the aorta was noted, nor had any been noted grossly.

Capillary engorgement with sickled erythrocytes was a prominent feature in the organs studied.

*Anatomical Diagnoses:* History of sickle cell anemia and two cerebral accidents. Sickle cell anemia with calcification and iron pigmentation of blood vessels; extensive endarteritis obliterans of splenic and cerebral arteries; siderofibrosis of spleen; anemic infarct of left cerebrum; subarachnoid and subdural hemorrhage; and congestion of lungs with pleural adhesions.

*CASE 2. Clinical History:* A 62 year old negress was admitted to the Duke Hospital on August 10, 1936. She had been married 30 years and had had 5 pregnancies with 5 healthy children. The patient had had severe parietal headaches at fortnightly intervals. In her early forties she was committed to an institution for a year or more because of some mental aberration. Her recovery apparently had been complete.

About 3 or 4 days prior to admission to the hospital she did not appear well although there was no specific complaint. Two days prior to admission she suffered severe pain in the lower back, abdomen and legs. She consulted her physician who found her temperature to be 100° F., the blood pressure 94/60 mm. Hg., and the pulse quite rapid. "Drops" were prescribed for the heart condition and another medicine of unknown composition was also prescribed. The patient complained less of pain and, at the same time, grew less communicative. During the following 12 hours she became unresponsive and was admitted to the hospital.

*Physical Examination:* The temperature was 38.8° C., pulse 108, respirations 24, and the blood pressure 80/60 mm. Hg. The patient was poorly developed and undernourished, and lay quietly in bed except for purposeless movements of the extremities. The chest was slightly increased in the anterior-posterior diameter. The lungs showed hyperresonance to percussion and the breath sounds were distant. Atelectatic râles were heard. The heart was

not enlarged and the spleen was not palpable. Moderate thickening of the peripheral vessels was noted. Neurological examination showed definite lower left facial weakness and hyperactive tendon reflexes. The abdominal reflexes were absent and a questionably positive Babinski's sign was present on the left side.

*Accessory Clinical Findings:* The hemoglobin was 58 per cent (9 gm.) Sahli, red blood cells 3,000,000, white blood cells 10,600 with 89 per cent polymorphonuclear leukocytes. The Wassermann and Kahn tests were negative. The plasma non-protein nitrogen was 61 mg. per cent. The urine showed a slight trace of albumin and gave a positive benzidine test. A lumbar puncture disclosed normal dynamics and clear spinal fluid which was negative for blood and globulin. A spinal fluid Wassermann test was negative. Roentgenogram of the chest showed the lungs to be clear. One sickle cell preparation was reported to be negative.

The patient became stuporous and finally comatose. Fever, pulse and respirations increased and incontinence of urine and feces developed. A white blood cell count at this time was 42,000. Treatment was mainly supportive. The patient expired quietly on the 3rd hospital day.

### *Autopsy Report*

*Macroscopic Examination:* The spleen weighed 250 gm., and measured 14 by 10 by 5 cm. It was covered with many adhesions and on section the pulp was red and extensive areas of infarction were present. One area of necrosis extended from the capsule through the entire diameter of the organ. The large blood vessels at the hilum showed no thrombi.

Minute hemorrhages were present in the subendocardium of the heart. Chronic passive congestion with central atrophy was noted in the liver. The aorta showed slight thickening of the intima.

The brain contained numerous minute hemorrhages (Fig. 7) from 1 to 2 mm. in diameter throughout both white and gray matter. Many softened areas were present about the hemorrhages. The blood vessels showed no abnormalities.

*Microscopic Examination:* Sickling of the erythrocytes was noted in a preparation made from freshly scraped splenic pulp.

The brain showed many large areas of focal necrosis, some of which were apparently unrelated to hemorrhage. Associated with the numerous hemorrhages in the brain substance were peculiar hyaline bodies resembling masses of fluid and blood cells within greatly congested capillaries. These bodies appeared somewhat like cells that had undergone extensive hyalinization. Similar hyaline bodies were seen in the infarcted areas of the splenic tissue.

The organs in general showed capillary engorgement with sickled erythrocytes.

*Anatomical Diagnoses:* Sick cell anemia; hemorrhagic splenic infarct; extensive focal hemorrhages in the lungs and heart, and especially the brain; lobular pneumonia; uterine polyp; and old fibrous pleural adhesions.

### DISCUSSION

Obliterative vascular changes and thrombosis in the spleen are definitely known to be a part of the pathological changes noted in sickle cell anemia. This has been described particularly in the vessels of the spleen by Diggs.<sup>9</sup>

In our 1st case the alterations of the cerebral and splenic blood vessels are similar. The blood vessels in the brain and spleen show perivascular hemorrhages, hyalinization of the media, and proliferation of the intima with occasional complete obliteration of the lumen and disruption of the internal elastic lamina, the latter containing iron and calcium deposits. This suggests the possibility that the cerebral and splenic vessels are injured by the same etiological agent.

The changes in the cerebral vessels in our 1st case were similar to those described by Winkelman and Eckel<sup>10</sup> in infectious diseases of infancy and childhood in that there were present sub-intimal proliferation with thickening and secondary degeneration of the intima, disruption of the elastic lamina, mild inflammatory reaction in the media and gradual disappearance of the muscle fibers with replacement by connective tissue. In addition, deposits of iron and calcium were found in the internal elastic lamina.

The splenic changes represent those found in siderofibrosis of the spleen which has been described and discussed by Diggs.<sup>9</sup> He states that the size of the spleen decreases, depending on the diminished vascularity of the organ, organization of hemorrhages and infarctions, and the replacement of the residual pulp by fibrous tissue.

In studying 3 spleens from cases of sickle cell anemia, frequent encrustations of iron and calcium in the form of rings throughout the fibrosed splenic pulp have been noted. This seems very likely to be the end result of fibrosis and degeneration of splenic vessels.

The sickled erythrocytes were easily demonstrated in unfixed

scrapings and in both Zenker and formalin-fixed tissues. The most characteristic sickled cells were narrow pointed rods and elliptical shaped cells, as mentioned by Diggs and Ching.<sup>11</sup> In studying the organs removed from negroes who had no history of symptoms of sickle cell anemia such pointed or elliptical shaped erythrocytes were not found in either Zenker or formalin-fixed tissues. There was an alteration in the shape of the erythrocytes, however, which consisted of decrease in size and some change in shape, oat or crescent, of the cells. No pointed rods were found.

#### SUMMARY AND CONCLUSIONS

1. A study of 2 cases of sickle cell anemia (1 in an adult and 1 in a child) has shown that the disorder may first become manifest through the appearance of signs and symptoms indicative of cerebral vascular disease. The clinical features in such cases lead to the diagnosis of either cerebral vascular thrombosis or intracranial hemorrhages.

2. The pathological changes seen in 1 of the cases establish the fact that in sickle cell anemia the large subarachnoid cerebral arteries may undergo gradual obliteration with final complete closure through the operation of a process identical with that which results in occlusion of the splenic arteries. This process is one of endarterial intimal proliferation and not of thrombosis.

3. In a 2nd case the autopsy showed that another vascular process, quite different from endarterial intimal proliferation, also occurs in sickle cell anemia. This process develops in connection with the small intracerebral vessels and may result in multiple focal necroses and hemorrhages in the brain, in contrast with the large infarcts that characterize the proliferative obstructive process in the larger arteries. The nature of this second process is not clear.

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## DESCRIPTION OF PLATES

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### PLATE 59

- FIG. 1. Microphotograph of spleen showing encrustations of iron and calcium, the former being in the form of rings, with diminished splenic pulp and increased connective tissue.
- FIG. 2. A splenic vessel showing fragmented elastic lamina, proliferation of the intima and perivascular hemorrhage.
- FIG. 3. Cross section of left middle cerebral artery showing partial occlusion and fragmented elastic lamina.
- FIG. 4. High power of inset (Fig. 3) showing fragmented internal elastic lamina with iron encrustations.



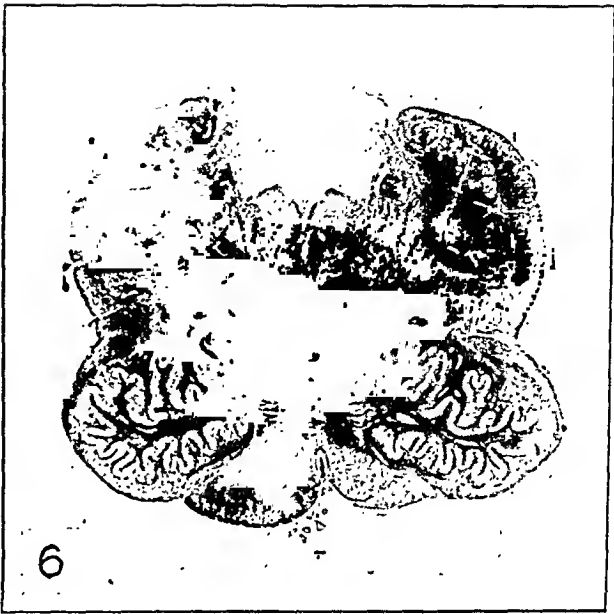
Bridgers

Cerebral Vascular Disease in Sickle Cell Anemia

PLATE 60

- FIG. 5a. Spleen showing siderofibrotic changes with marked wrinkling of the capsule.
- FIG. 5b. Section of spleen showing large area of hemorrhage and greatly scarred pulp.
- FIG. 6. Microphotograph of a section of the medulla showing marked difference in size of the right and left pyramidal tracts, the former being markedly degenerated.
- FIG. 7. Cross section of adult brain showing multiple petechial hemorrhages throughout.





Bridgers

Cerebral Vascular Disease in Sickle Cell Anemia

PLATE 61

FIG. 8. Case 1. Inferior surface of brain showing subarachnoid hemorrhage in the form of a blood clot with inequality in the size of the frontal lobes.

FIG. 9. Case 1. Cross section of posterior aspect of brain showing marked atrophy of left cerebral cortex, dilated ventricles, petechial hemorrhages and a large hemorrhage in the right hemisphere.



Bridgers

Cerebral Vascular Disease in Sickle Cell Anemia



# PATHOLOGICAL CHANGES FOLLOWING THERAPEUTIC HYPERTHERMIA \*

## REPORT OF A CASE

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In view of the current vogue that therapeutic hyperthermia enjoys for a wide variety of ailments, it is appropriate to take stock of the more serious complications that may follow its use even under proper management as a hospital procedure and with careful selection of patients. In a preliminary survey<sup>1</sup> of hyperpyrexia produced by physical agents, the Council on Physical Therapy of the American Medical Association stated in 1934 that 29 deaths following fever treatment had been reported by physical therapists in response to its questionnaire. Since then at least 16 additional fatalities have been reported.<sup>2-12</sup> Moreover, there is good reason to believe that there have been a considerable number of other fatal accidents which for one reason or another have not been recorded in the current literature. Although it is in only a few of these cases that complete autopsies have been performed, sufficient data have accumulated to permit a satisfactory discussion of at least the morbid anatomical changes that may result from fever therapy, by whatever physical means induced. While there appears to be considerable variation in the changes observed in individual cases, certain structures, notably the brain, liver and blood vessels, seem particularly susceptible to injury of a type to be described presently, and with sufficient frequency to be regarded as characteristic.

The writer has had occasion to perform an autopsy on a patient who developed uncontrollable hyperpyrexia (109° F.), coma, and respiratory failure in the course of the third of a series of hyperthermia treatments for infectious arthritis of the fingers. The patient died approximately 35 hours after fever was initiated. The method of inducing hyperthermia was that of hot bath followed by blanket pack. The clinical course will be related in some

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detail, not only because it is dramatic but because it is essential to the interpretation of the pathological changes that ensued. The fact that the patient was a comparatively young man of good general physique and in good health, except for the arthritis, makes it possible to ascribe the pathological changes observed to the hyperthermia *per se*. The changes found at autopsy are of particular interest, furthermore, in that they demonstrate certain interesting vascular lesions occurring in the brain, kidneys and spleen, hitherto undescribed in connection with fatalities following fever therapy.

### REPORT OF CASE \*

*Clinical History:* The patient, a white male 36 years of age, robust of build and in good general health, was admitted to the Hospital for Joint Diseases on Sept. 25, 1936, in order to receive fever therapy for infectious arthritis of the fingers of undetermined etiology. He had had 2 previous treatments for the same complaint without ill effect and with some symptomatic improvement. The method of inducing hyperthermia was that of hot bath followed by blanket pack. The physical examination done before treatment was essentially negative except for the arthritis. The blood pressure was 112 systolic, 80 diastolic.

Fever treatment using bath and blanket pack was begun at 1 P.M., the temperature being raised from 97.8° F. (initial) to 105.2° F., by mouth, within 35 minutes. The patient's pulse varied from 134 to 148, and the temperature fluctuated between 104.6° F. and 105° F. for the next 4 hours. One ¼ grain dose of morphine was given at 1.45 P.M. and this was repeated at 5.30 P.M. because of excessive excitement. At 6.15 P.M. the temperature suddenly rose to 106° F. (pulse 138). At 6.30 P.M., with the temperature still at 106° F., the patient was uncovered to expose both shoulders, but the temperature nevertheless continued to rise steadily to 107.4° F. within the next 15 minutes. Treatment was stopped immediately; the patient was rapidly unpacked and received coramine hypodermically by way of stimulation. The temperature dropped to 106.2° F. but again rose promptly to 107° F. (pulse 168) and despite cool sponging continued to climb to 109° F. (rectal). An ampule of sodiocaffeine benzoate was administered intravenously. At 7 P.M. the patient lapsed into unconsciousness and his pulse was rapid. By 7.25 P.M., after a cool enema containing 30 grains of aspirin, the temperature dropped sharply to 100° F. (rectal). At 7.35 P.M. the temperature again rose to 104.4° F. (rectal). At 8 P.M. an intravenous hyperdermoclysis of normal saline was begun. The temperature fluctuated between 100° F. and 105° F. during the next 2 hours. Caffeine and coramine were again administered. The patient vomited about 8 ounces of dark brown material. A Levine tube was passed to evacuate the stomach and gastric lavage was instituted with 5 per cent sodium bicarbonate. At 10.35 P.M. the patient's respirations stopped but he responded to artificial respiration. The patient again received caffeine and coramine intravenously, intracardiac injection of adrenalin, and also oxygen and carbon dioxide by nasal catheter.

\* The writer wishes to express his indebtedness to Dr. M. Dinnerstein for permission to include the clinical data in this case.

With the aid of these emergency supportive measures, the patient lasted through the night. At 5 A.M. the next morning, with the patient in an oxygen tent and still comatose, clonic twitchings of the left lower extremity were observed and it was noted that he had a right hemiplegia, including the face, of flaccid type. At 10 A.M. that morning he was seen by a neurological consultant, who tapped about 20 cc. of spinal fluid under increased pressure. Laboratory examination of this fluid showed increased globulin content and a cell count of 14 white blood cells and 9 red blood cells per cmm. The spinal fluid Wassermann was negative. The urine specimen at this time contained albumin (++) , casts, leukocytes, and an occasional red cell.

Despite repeated stimulation with coramine, adrenalin, digitalis and caffeine, the patient remained stuporous and finally expired at 12.20 A.M. (Sept. 27, 1936), 35 hours after hyperthermia was started and approximately 29 hours after the onset of coma and collapse.

### POSTMORTEM EXAMINATION

Autopsy was performed 2 hours after death. The body was that of a robust, well developed adult white male. The body was still warm and rigor mortis had not yet set in. External examination did not reveal any obvious swellings of the finger joints. There was marked purplish mottling of the face and trunk, and considerable postmortem lividity. No jaundice or edema was noted.

The panniculus adiposus was moderately thick. The peritoneal surfaces were everywhere smooth and glistening, except for some fibrous bands extending from the cecum to the anterior abdominal wall at the site of an old appendectomy scar. The intestines showed no distention whatsoever and no evidence of hemorrhage. The diaphragm on the left side appeared to be somewhat elevated, apparently in consequence of gastric dilatation.

There was no fluid in the pleural cavities. The upper lobes of the lungs felt hypercrepitant, while the lower lobes, particularly the left, showed large purplish areas of atelectasis. On section the lungs had a mottled, dark reddish purple appearance and exuded an inordinately large amount of venous blood. There was no gross evidence of bronchopneumonia, although the bronchi were somewhat congested and contained frothy pinkish fluid. The pulmonary arteries and veins appeared normal.

The pericardium contained a small amount of clear fluid. The heart showed slight enlargement due to dilatation of its chambers. The valves showed nothing unusual except for occasional lipoid plaques. The myocardium was firm and dark red in color. There

were a few subendocardial petechial hemorrhages in the left ventricle. The ostia and lumens of the coronary arteries were widely patent.

The liver was slightly enlarged, soft and smooth, and was a yellowish brown color. On section the lobular architecture was found clearly outlined. Scattered throughout the liver were small, focal yellowish areas which appeared to be fat, but which were nevertheless somewhat suggestive of focal areas of necrosis. The gall bladder was slightly distended. The bile ducts and portal veins showed nothing noteworthy.

The spleen was soft and somewhat enlarged. There was a circumscribed, slightly elevated subcapsular area of infarction, about 2 cm. in diameter, on its medial aspect. The splenic pulp had a dark red appearance and could not be scraped away easily. The malpighian follicles were quite distinct.

The pancreas showed nothing noteworthy.

The adrenals were of average size. They presented a yellow lipid cortex and some autolysis of the medulla.

The kidneys were of normal size and their capsules stripped with ease revealing smooth, slightly lobulated reddish surfaces. The latter showed several dark red mottled areas, about 1 cm. in diameter, which on section appeared to be fresh infarcts. The cortex and medulla had indistinct markings. The ureters and urinary bladder presented nothing unusual. The prostate was small and on section showed no evidence of inflammation.

The stomach was moderately distended and contained turbid dark fluid. The gastric mucosa was markedly congested and revealed a few petechial hemorrhages but no gross evidence of gastritis. The duodenum was not unusual in appearance. The remainder of the gastro-intestinal tract presented nothing striking, *i.e.* no evidence of ulceration, necrosis or hemorrhage.

On removal of the calvarium the brain was noted to be voluminous and quite heavy. The inner aspect of the dura had a thickened, reddish hemorrhagic appearance. The sulci of the cerebral cortex were somewhat flattened and the surface of the hemispheres showed intense venous engorgement and considerable edema. There were also several superficial reddish areas of hemorrhage, about 1 to 2 cm. in diameter, noted beneath the pia.

The cortex of the cerebral hemispheres was edematous, quite



soft and friable. Section of the brain revealed innumerable focal punctate hemorrhages distributed throughout the gray matter of the cortex. These were sharply limited to the cortical gray matter and were most prominent in the depths of the sulci (Fig. 1). They frequently involved several contiguous sulci, but on the whole their distribution was distinctly patchy. The punctate hemorrhages were more extensive in the left hemisphere than in the right. Microscopic sections indicated that most of the hemorrhagic areas still contained well preserved red cells, but in places one could observe beginning laking with blood pigment deposits (Fig. 2). There were no hemorrhagic lesions in the white matter of the hemispheres. In the left internal capsule, however, there was an irregular area of hemorrhage and softening approximately 1 to 2 cm. in diameter (Fig. 4). The pons, medulla and cerebellum were sectioned serially, but no evidence of hemorrhage, softening or conspicuous edema was observed. The spinal cord could not be examined.

*Anatomical Diagnoses:* Status (35 hours) after therapeutic hyperthermia for arthritis: multiple punctate hemorrhages in gray matter of cerebral cortex; hemorrhage in left internal capsule; thrombosis of venules and capillaries in cerebral cortex and internal capsule; cerebral congestion and edema; focal necrosis of interlobular arteries of kidneys and splenic artery branches; infarcts of kidneys and spleen; severe hepatic degeneration and edema; subpleural and subendocardial petechial hemorrhages; pulmonary congestion, hemorrhage and edema; and partial atelectasis of the lower lobes of both lungs.

Microscopic sections confirmed the gross changes as outlined in the anatomical diagnoses. The significant histological observations will be indicated in connection with the ensuing discussion.

## DISCUSSION

The following discussion of the anatomical changes occurring after therapeutic hyperthermia is based largely on the present writer's own observations, but also on the information afforded by 9 autopsies reported within the past few years. These published autopsy reports<sup>2-4, 8, 9, 11, 12</sup> have, in most instances, been presented briefly, often without important details or illustrative microphotographs, but they serve nevertheless to indicate in a

general way the principal complications and sequelae of therapeutic hyperpyrexia.

*Pathological Changes in the Brain:* It is usually in the brain that the most extensive and consequential damage is encountered after therapeutic hyperthermia. To judge by the recorded fatalities, cerebral edema and conspicuous congestion of the meningeal vessels are constantly present. In our case also, the brain was swollen and quite heavy and the surface of the hemispheres showed intense venous engorgement. There was also a pressure conus of the cerebellum.

Another frequent observation is meningeal hemorrhage, which seems to be attributable to extreme venous and capillary vasodilatation. Specifically, rupture of engorged venules probably occurs, with consequent perivascular hemorrhages. Thus, the inner aspect of the dura may have a thickened, diffusely hemorrhagic appearance, or on the other hand, the subdural hemorrhages may be less prominent and focal in distribution. Small extravasations of blood beneath the arachnoid and pia mater have also been noted. Equally conspicuous and of greater significance is the presence in our case of innumerable punctate hemorrhages, irregularly distributed throughout the gray matter of the cerebral cortex, as illustrated in Figure 1. Microscopic examination shows most of these extravasations of blood to be situated around congested venules and capillaries. Perivascular hemorrhages have also been described<sup>2,11</sup> in the basal ganglia and in the cerebellum. Moreover, hemorrhage and softening may occur in the internal capsule (explaining the development of hemiplegia) as in the case reported here. Histological examination of sections of the hemorrhagic portion of the internal capsule reveals numerous perivascular blood extravasations, many of which are limited to the Virchow-Robin spaces while others appear to be somewhat more extensive (Fig. 4).

Associated with these hemorrhagic phenomena there is pronounced cellular degeneration and even necrobiosis in the gray matter of the cerebral cortex. Microscopically the nerve cells of the more hemorrhagic portions of the cortex (Fig. 2), in contrast with the relatively well preserved zones (Fig. 3), show considerable alteration. Some nerve cells reveal ischemic changes, while others are shrunken and deeply pigmented. Indeed, many cells

have completely disappeared, leaving wide bands of necrobiosis which terminate more or less abruptly where the preserved cortex begins again. In connection with the shrinkage of cerebral cortical cells after hyperthermia there is, as Yannet and Darrow<sup>13</sup> have shown, a loss of intracellular fluid into the extracellular tissue, resulting in increased concentration of intracellular electrolytes.

Furthermore, there were vascular lesions in our case within the hemorrhagic portions of the cerebral cortex and internal capsule, which in themselves are of considerable interest. Microscopic examination of sections of the affected portions of the cortical gray matter reveals a number of thrombosed venules which show evidence of severe injury, if not actual partial necrosis of their walls, as indicated by loss of normal structure and continuity, as well as by intramural and perivascular infiltration with polymorphonuclear leukocytes. The lumen of the venule shown in the accompanying microphotograph (Fig. 5) is partially filled with homogeneous material possessing the staining properties of fibrin. Numerous small venules and capillaries in the hemorrhagic area of the internal capsule reveal similar evidence of injury. In view of the intramural leukocytic reaction, these vascular lesions must be regarded as occurring ante mortem. However, they cannot be held entirely responsible for the cellular changes in the cortical gray matter, since fairly extensive necrobiosis of cortical nerve cells has been noted following therapeutic hyperthermia without the presence of fibrin thrombi in venules.

The presence of conspicuous hemorrhages in one or another part of the brain has led some observers to describe the condition of this organ erroneously as a hemorrhagic encephalitis. The use of the term encephalitis in this connection is inaccurate, since microscopic examination does not disclose any evidence of inflammation associated with the hemorrhages. Our knowledge of the subject would be more enhanced if, in the future, the actual pathological changes in the brain in any given instance were described objectively in some detail, rather than an attempt made, for the sake of convenience, to pigeonhole them in a category to which they do not belong.

In our case a history was elicited of an alcoholic excess prior to hyperthermia treatment, and that naturally raises the question of the possible contributory rôle of alcohol in the production of

cerebral injury following hyperpyrexia. This possible association is further suggested by the observation, well known in large municipal hospitals, that heat stroke is prone to develop in moderate or heavy drinkers. Of course, alcoholism *per se* does not give rise to the cerebral lesions described in the case reported here. However, chronic alcoholism is known<sup>14</sup> to be capable of producing cerebral edema, degeneration of cortical ganglion cells, and endothelial injury sometimes resulting in petechial hemorrhages in the dura and in the midbrain. Acute alcoholic intoxication may likewise cause cerebral edema and also marked vasodilatation. It seems justifiable therefore to add alcoholism to the list of more obvious contra-indications which make artificial fever therapy hazardous: namely, advanced age or marked debility, cardiovascular disease, renal insufficiency, tuberculosis, diabetes, and so on. Furthermore, it seems a reasonable precaution to advise patients receiving a course of hyperthermia treatments to refrain from alcoholic indulgence during the interval preceding each treatment.

*Vascular Lesions in Other Organs:* In addition to the vascular lesions described in the brain there were also changes in the kidneys and in the spleen. Such lesions have apparently not been observed by others who have dealt with the question of the pathological changes in these cases. In both kidneys in our case there were multiple, subcapsular, wedge-shaped areas of necrosis, and in the spleen there was a similar area of necrosis. These were indistinguishable from infarcts with respect to location, shape and general appearance, and were not to be confused with the focal areas of toxic necrosis that may occur in the kidneys and other viscera, after severe burns for example. The latter areas are usually irregular in outline, smaller in size, not necessarily peripheral, and have a random distribution without reference to vascular supply. The necroses were apparently not due to embolization, nor was any possible source for emboli found in the heart, aorta or arterial system. Furthermore, the foramen ovale was closed. The blood vessels of the kidneys and spleen moreover showed no evidence of previous thrombosis.

The natural inference therefore is that the renal and splenic infarcts were the result of focal necrosis of interlobular arteries and splenic artery branches respectively. Indeed, that is what was

indicated by the histological sections. Figure 6 (inset) shows a necrotic interlobular artery in the vicinity of one of the renal infarcts. The wall of this artery, higher magnification of which is shown in Figure 7, was necrotic and infiltrated by polymorphonuclear leukocytes. Within its lumen there were irregular aggregates of homogeneous, reddish staining material resembling fibrin and agglutinated erythrocytes. The artery shown in the illustration is probably too small to account for the renal infarct, but it illustrates the type of arterial lesion that must have developed in renal arteries of somewhat larger caliber. Sections of the spleen showed a number of similarly necrotic small splenic artery branches in the vicinity of the infarct. Weigert and Van Gieson stains of affected arteries demonstrated disappearance and disruption of the elastica and collagen fibers. Examination of the pulmonary arteries showed focal areas of altered staining with occasional polymorphonuclear leukocytes within their walls, which suggested vascular injury similar to that observed in the brain, kidneys and spleen, but of lesser extent.

*Damage to Liver:* Of all the pathological changes observed in fatalities following therapeutic hyperthermia, damage to the liver is secondary in importance only to the injury to the brain. Histological evidence of degeneration of varying degree in the liver appears to be frequent. In connection with the more severe reactions necrosis may develop, and indeed this was noted in 4<sup>3, 8, 12</sup> of the 6 reported fatalities in which the status of the liver was mentioned. Also in the case herein described the liver cells showed microscopically extensive vacuolization and hyaline droplet degeneration (Fig. 8). Areas of necrobiosis were likewise present, as evidenced by pyknosis and disappearance of nuclei, with effacement of cytoplasmic detail. A sudan III stain for fat showed the liver cells to contain numerous fat droplets. In addition to these degenerative changes there was also hepatic edema, as indicated by separation of the sinusoidal walls from the liver cell cords in many places and the presence of granular material within these pericapillary spaces.

That damage to the liver may also occur in non-fatal reactions to therapeutic hyperthermia is indicated by the reports of jaundice<sup>9, 15, 16</sup> developing as a complication of treatment. Warren and coworkers,<sup>16</sup> for example, noted the appearance of jaundice

after prolonged fever therapy in 7 patients. One may reasonably inquire whether some of these individuals may not subsequently develop nodular cirrhosis of the liver, but that question cannot as yet be answered.

*Pulmonary Changes.* The usual anatomical changes in the lungs after fatal reactions to therapeutic hyperthermia are marked congestion, edema and alveolar hemorrhage. These changes were noted in most of the available reports in which the lungs were described histologically. In our case, too, microscopic examination showed extreme engorgement of the pulmonary arteries and alveolar capillaries, as well as extravasation of blood into the alveoli, apparently as a result of capillary rupture. These findings are apt to be more pronounced in the lower lobes, where hypostasis also favors development of partial atelectasis. In patients who survive for several days or more bronchopneumonia tends to develop in the hemorrhagic areas.

*Changes in the Suprarenal Glands:* The published reports of hyperthermia fatalities in which the status of the adrenals is given do not indicate that there is any significant injury to the suprarenal glands. Also in the case reported here there were no striking histological changes. It should be mentioned, however, that hemorrhage into the suprarenals has been noted<sup>2</sup> and that degenerative changes in the adrenal cortex have been described.<sup>2,9</sup> The latter, however, have not been clearly distinguished from postmortem changes, which may proceed rapidly in the suprarenal glands after febrile states.

*Pathological Changes in Other Organs:* To complete this résumé of the pathological changes following therapeutic hyperthermia, the changes noted in the other organs will be briefly mentioned. The heart often shows epicardial or endocardial petechial hemorrhages. The kidneys may show hyaline droplet degeneration of the epithelium of the convoluted tubules, hyaline tubular casts, and conspicuous congestion of the glomerular tufts. The spleen in the case reported here showed, microscopically, intense hyperemia of the pulp and fibrin thrombi within pulp venules. In the gastro-intestinal tract dilatation of the stomach as well as congestion and petechial hemorrhages of the gastric mucosa were noted. Hemorrhages in the jejunum and ileum have also been described.<sup>9</sup> Severe gastro-intestinal hemorrhage has been observed in a patient

who died of acute necrosis of the liver following therapeutic hyperthermia.<sup>12</sup>

It should be pointed out that the extent and distribution of the pathological changes in any given instance depend not only on the severity of the injury, but also on the length of the survival period. For example, the histological changes described in the brain take more than a few hours to develop. Moreover, if the cerebral insult is so great that it leads rapidly to fatal edema of the brain, one may not have the opportunity of observing changes in the liver or the vascular lesions which have been described. Thus, in an interesting case cited by Kopp and Solomon,<sup>9</sup> jaundice did not manifest itself until the 3rd day following hyperthermia. By the same token the pathological changes described in the hitherto reported fatalities may not necessarily represent a complete picture of the possible complications and sequelae of hyperthermia. As the survival period is lengthened and as more pathological material is studied, other evidences of tissue injury and response may manifest themselves.

#### COMMENT

In attempts to explain the pathogenetic mechanism of the changes that have been noted after therapeutic hyperthermia, various hypotheses have been suggested. Hartman<sup>11</sup> maintains from his own experimental data and also on theoretical grounds, that anoxemia is a paramount factor in the development of cerebral injury after hyperpyrexia treatment. However, it is questionable whether it is correct to attribute the cerebral damage entirely or even largely to anoxemia, to the exclusion of other, probably more weighty factors. Another hypothesis, particularly stressed by Kopp and Solomon,<sup>9</sup> is that of vasomotor shock or collapse. Still other observers have pointed out that the pathological changes bear some similarity to those observed in individuals who succumb to heat stroke. Dehydration, anhydremia and electrolyte depletion are all physiological effects of severe hyperthermia, which may influence and modify the clinical course. The use of certain sedative drugs, for example sodium amytal and morphine, has also been suggested as a possible contributory factor. Furthermore, the convulsions that frequently occur in severe hyperthermia reactions may be responsible for cerebral hemor-

rhages. It may readily be seen therefore that the problem of explaining the pathological changes that may follow therapeutic hyperthermia induced by physical means is a complex one owing to the interplay of many factors whose relative importance is difficult to evaluate.

A note of caution is decidedly in order with regard to the serious but not necessarily fatal complications of therapeutic hyperthermia. Physical therapists conducting active fever therapy services generally admit<sup>9, 17, 18</sup> to an appreciable number of patients who in the course of treatment go into so-called "shock" or "circulatory collapse," associated usually with uncontrolled hyperpyrexia, but whom they are able to resuscitate by immediate cessation of treatment and emergency supportive measures. These patients frequently manifest delirium, mania, disorientation, convulsions, epileptiform seizures or coma. They may even develop loss of bladder and rectal sphincter control, transient hemiplegia, facial palsy or aphasia. The exponents of fever therapy are inclined to minimize the significance of such untoward episodes. Even neurologists and psychiatrists<sup>19</sup> are prone to pass lightly over these manifestations of brain injury as transitory neurological changes. The possibility, however, that such individuals may actually sustain organic lesions in the brain, and perhaps in other viscera, cannot be dismissed. Possibly, therefore, our attitude should be a more guarded one for the present, pending follow-up observation of such individuals for hyperthermia sequelae.

#### SUMMARY

A description is given of the changes observed at autopsy in a case of uncontrollable hyperpyrexia (109° F.) ensuing upon hyperthermia treatment for arthritis of the finger joints. The hyperpyrexia (which developed in the course of the third of a series of treatments) was associated with coma and respiratory failure, and the patient died about 35 hours after the fever was initiated. In this case the significant pathological changes were the following: (1) multiple punctate hemorrhages and necrobiosis in the gray matter of the cerebral cortex; (2) hemorrhage in the left internal capsule; (3) thrombosis of venules and capillaries in the cerebral cortex and internal capsule; (4) cerebral congestion and edema; (5) infarction of kidneys and spleen; (6) marked



hepatic degeneration and edema; and (7) pulmonary congestion, hemorrhage and edema.

The changes found in this case have been correlated with, and discussed in relation to, those in 9 cases previously recorded in the literature. Certain of the changes seen in the case reported here, notably the vascular lesions, have not hitherto been described in connection with fatalities following fever therapy. Specifically there seems to be no previous description of the thrombosis of venules and capillaries in affected portions of the brain, and of the infarcts in kidneys and spleen, apparently due to focal necroses of small arterial branches of these organs.

The principal complications and sequelae of hyperthermia — and especially its effects upon the brain, blood vessels and liver — are indicated. Attention is also drawn to the fact that the reactions to therapeutic hyperthermia are sometimes serious even when they are not fatal.

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## DESCRIPTION OF PLATES

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### PLATE 62

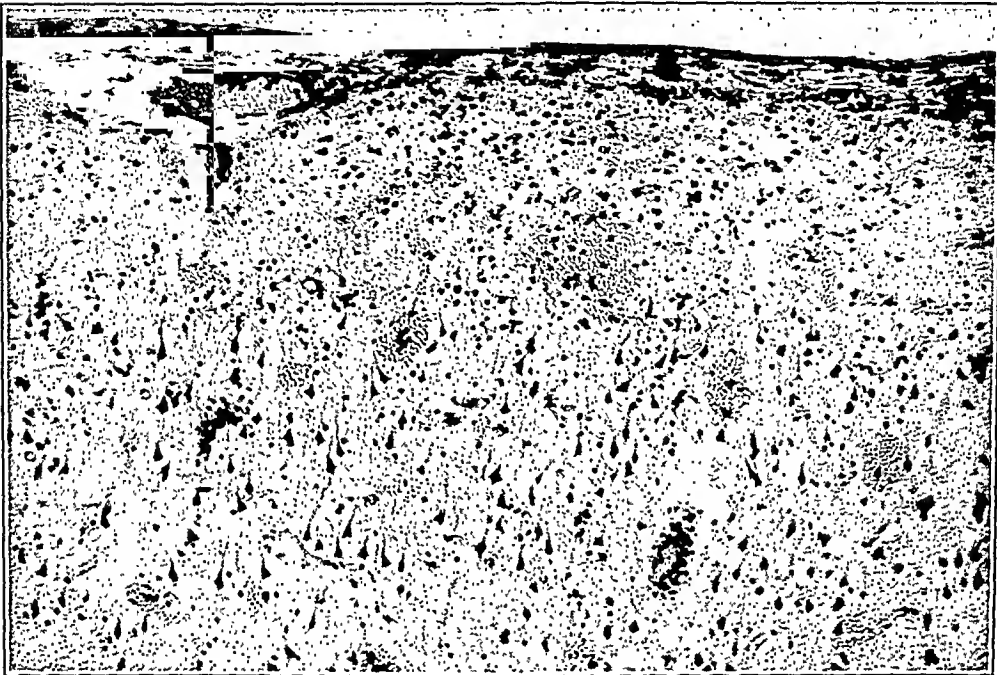
FIG. 1. Photograph of section through the left cerebral hemisphere showing multiple punctate hemorrhages limited to portions of the gray matter of the cortex. Note also the hemorrhagic appearance of the leptomeninges.

FIG. 2. Microphotograph of hemorrhagic area in gray matter of cerebral cortex showing numerous small perivascular extravasations and extensive cellular degeneration. Toluidine blue stain.  $\times 125$ .

FIG. 3. Microphotograph of relatively well preserved area of cortical gray matter for contrast with Fig. 2. Note engorgement and vasodilatation of meningeal vessels. Toluidine blue stain.  $\times 125$ .



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Lichtenstein

Changes Following Therapeutic Hyperthermia

PLATE 63

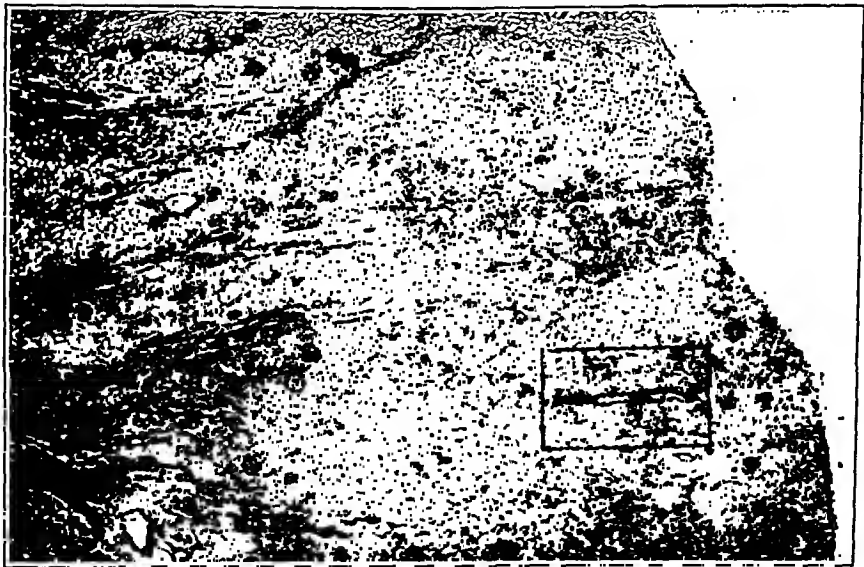
- FIG. 4. Microphotograph of an area of hemorrhage and softening in the left internal capsule. Note the perivascular extravasations and the thrombosed venule.  $\times 85$ .
- FIG. 5. Microphotograph of venule in hemorrhagic portion of cortical gray matter, showing fibrin thrombus and intramural infiltration with polymorphonuclear leukocytes.  $\times 250$ .
- FIG. 6. Microphotograph of one of the renal infarcts. Note the necrotic interlobular artery in inset.  $\times 25$ .



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Lichtenstein

Changes Following Therapeutic Hyperthermia

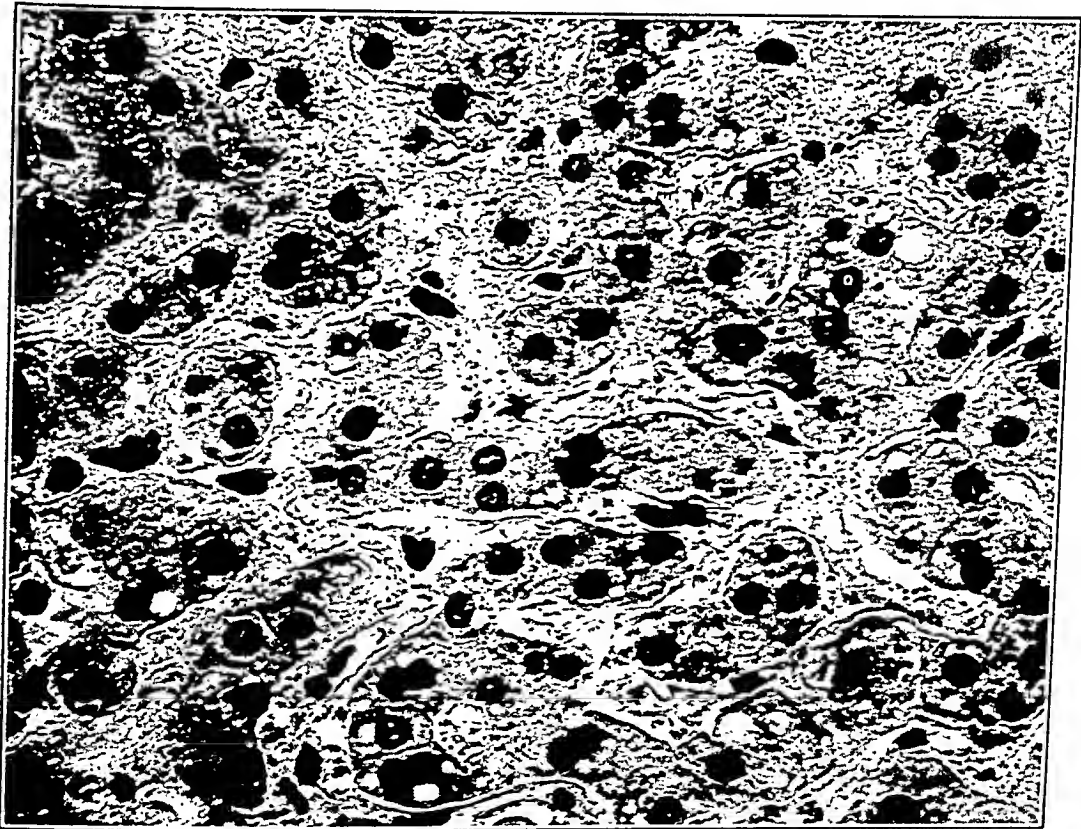
PLATE 64

FIG. 7. Higher magnification of necrotic interlobular artery illustrated in Fig. 6.  $\times 100$ .

FIG. 8. Microphotograph of liver showing severe degenerative changes of liver cells and evidence of hepatic edema.  $\times 600$ .



7



8





# THE LYMPHOID NODULES OF HUMAN BONE MARROW \*

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## REVIEW OF THE LITERATURE

A survey of the literature shows that Dominici<sup>1</sup> in 1902 observed focal collections of lymphocytes in the bone marrow. Longcope<sup>2</sup> described these nodules in the marrow as a characteristic occurrence in typhoid fever and also noted small foci in 2 cases of pneumonia. Hedinger<sup>3</sup> demonstrated a number of large follicles with large germinal centers in the femoral marrow of a 1½ year old idiot with so-called "status lymphaticus." Dickson<sup>4</sup> stated that nodules of small lymphocytes are scanty in normal marrow. Oehme,<sup>5</sup> in a series of 23 cases of rickets, and in 2 other cases, described, in otherwise normal appearing red marrow, follicles with germinal centers in 12 cases and others without germinal centers in 2 cases. The majority of Oehme's cases were infectious and all were infants or children. He was undecided as to whether these follicular structures were normal or not. Such a high incidence of follicles with germinal centers has not as yet been confirmed.

In 1915 Askanazy,<sup>6</sup> in a study of the marrow from different regions of the femur in a series of 126 unselected cases, most of which were adults, described scattered lymphoid nodules in 33 per cent. These nodules varied in size from 201 to 542 microns in diameter. They were composed mostly of small lymphocytes, intermingled at times with myeloid cells, and each follicle had a reticular framework with a central or eccentric arterial capillary. Only 1 case presented germinal centers and local lesions did not account for the presence of the foci of lymphocytes. These foci were more frequent in adult individuals over 40 years of age. The author concluded that lymphoid nodules in the bone marrow cannot be considered abnormal but that an excessive number and many germinal centers may be a pathological phenomenon.

Von Fischer,<sup>7</sup> in a detailed histological study of the marrow of

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the humerus in a series of 61 unselected cases, and from the ribs and vertebra in 11 of the same series of cases, confirmed and extended Askanazy's findings. Her cases were mostly in adults. She emphasized the association of these lymphoid nodules with active red marrow. It is to be noted that she found lymphoid nodules in the vertebral marrow in 9 of the 11 cases in which this marrow was studied. Mayer and Furuta<sup>8</sup> demonstrated lymphoid nodules in 15 per cent of 58 essentially morphologically different cases and agreed with Askanazy and von Fischer as to their significance. It is to be noted that infection and inflammatory lesions in the various organs are frequent in such varied material as that studied by the latter authors, as will be pointed out later.

Ziegler,<sup>9</sup> Karsner<sup>10</sup> and others described focal aggregations of lymphocytes in the marrow in pernicious anemia. Custer,<sup>11</sup> Darling and co-authors<sup>12</sup> and others reported them to be present in cases of agranulocytosis. Custer, in commenting on the dense focal accumulations of lymphocytes in the marrow in 9 of his 11 cases of agranulocytosis, on the basis of the presence of considerable numbers of large, pale, delicately staining cells in the central portion of the lymphoid masses, wondered whether they were true lymph follicles or merely folliculoid accumulations in response to focal degeneration. He stated that the nodules are seen infrequently in the marrow in other types of disease.

#### MATERIALS AND METHODS

The material for this study consisted of a series of 265 unselected cases. All were adult individuals. Those showing primary diseases of the hemopoietic system were excluded. Marrow from the vertebra, sternum, humerus, femur and tibia in the first 122 cases, and from the vertebra and upper third of the shaft of the humerus in the remaining 143 cases, was studied. In addition, marrow from a second series of 23 adults, designated henceforth as the control series, was studied. Only cases with no significant inflammatory lesion demonstrable anywhere in the various organs of the body were selected for this second series. Marrow from the lumbar vertebra and upper third of the shaft of the humerus in all cases was studied. In 11 cases marrow from the lower half of the shaft of the femur was utilized for study. In a strict sense inflammatory lesions could not be considered eliminated from all cases of this

series since, for example, the presence or absence of dental or accessory nasal sinus infections was not commonly confirmed. However, the series as a whole represented one in which the incidence and extent of inflammatory lesions were reduced to a minimum. In 10 of these 23 cases, which were traumatic and where all the individuals died within 6 hours after injury, only the bone marrow was studied histologically. The remaining organs were studied only in gross. All other cases in both series were completely reviewed, both in gross and histologically.

The section technic patterned after the recommendation of Custer<sup>13</sup> was employed. Both hematoxylin-eosin and Maximow's azure II eosin and alum hematoxylin stains were used. In addition to these, consecutive sections from the same blocks of tissues from selected cases were stained with Foot's modification of Bielschowsky's method for reticulum and Mallory's phosphotungstic acid hematoxylin method for fibrin.

#### HISTOLOGICAL EXAMINATION

Since the lymphoid nodules studied in each series were quite similar, they may be described together. They were roughly oval in shape and at low magnification stood out in sharp contrast with the surrounding areas of granulopoiesis and erythropoiesis (Fig. 1). In addition to the oval shaped nodules, other areas appeared in sections as elongated structures with narrow diameters. The margins were slightly irregular and extended outward for short distances between adjacent fat cells, merging imperceptibly with the surrounding myeloid tissue. Fat cells were not noted within the nodules. The lymphoid nodules in the first series varied from 560 by 450 microns to 90 by 90 microns. Those in the control series varied from 525 by 450 microns to 90 by 90 microns, and there were also variations between these figures. From 1 to 3 nodules were seen in one low power field, but usually only 1. In a single section only 1 to 4 of these nodules were usually present. Ordinarily they were isolated and widely scattered. Here and there the structures contained capillaries filled with erythrocytes. Many of the nodules in the sections contained a centrally or eccentrically placed blood vessel with an outside diameter of 14 to 20 microns with the thickness of the wall equal to or slightly less than the diameter of the lumen. These were apparently precapillary arterioles.

The cells of the lymphoid nodules were made up almost exclusively of small round cells (Fig. 2), evenly and symmetrically placed, which were morphologically identical with the small lymphocytes of lymph nodes, as determined with the technic employed. A few medium sized to large lymphocytes were usually present. Occasional myelocytes, normoblasts, plasma cells and macrophages were seen, usually at the peripheries of the nodules. Rarely myelocytes were numerous but the lymphoid nodules retained their histological identity. Cells with an oval to fusiform, pale vesicular nuclei and ill defined cytoplasm were scattered here and there. A supporting structure composed of a network of branching argentophil fibrils enclosing groups of cells and occasional single isolated cells was seen (Fig. 3). The nodules were variously located in relation to other structures of the marrow. Occasionally they occurred adjacent to a small artery, venous sinusoid, trabecula of bone, or in the midst of myeloid tissue. The nodules in the material studied were generally located in active marrow, although the hemopoietic cellularity of the marrow at times was slight. They were not found in otherwise wholly fatty marrow.

No activity, as judged by mitosis or increasing number of medium sized and large lymphocytes with increasing basophilia of the cytoplasm, or follicles with germinal centers were seen. There was no constant predominance of any particular cell type in the region at the periphery of the nodules. Other than hyaline and fibrinoid changes within an occasional nodule, there was no evidence that the cells composing the nodules were a response to focal degeneration.

#### INCIDENCE OF LYMPHOID NODULES IN THE MARROW AND THEIR RELATION TO INFLAMMATORY LESIONS ELSEWHERE IN THE BODY

Table I shows the incidence of inflammatory lesions in the first

TABLE I  
*Relation of Lymphoid Nodules in the Marrow to Inflammatory Lesions*

Material	Total number of cases	Number of cases with inflammatory lesions	Incidence
Marrow with lymphoid nodules	78	75	% 96
Marrow without lymphoid nodules	187	169	90

TABLE II  
Data from 23 Cases of the Control Series

Number of case	Age	Sex	Diagnosis	Degree of hemopoietic cellularity			Lymphoid nodules present	Duration
				V	H	F		
	yr.			%	%	%		
1	55	F	Traumatic injuries	75	55	..	V. H.	1 hr.
2	Adult	M	Traumatic injuries	70	0-50	0-30	H. F.	1 hr.
3	Adult	M	Traumatic injuries	75	50	0	H.	1 hr.
4	55	M	Traumatic injuries	75	0-50	0-1	V. H.	1 hr.
5	33	M	Traumatic injuries	75	0-25	0-1	F. H.	6 hrs.
6	68	M	Cerebral hemorrhage	5-75	0	..	V.	17 hrs.
7	80	M	Hypertension, chronic cardiac failure	80	0-1	0-1	V.	3 mos.
8	73	M	Cerebral hemorrhage	75	0-25	..	V.	4 hrs.
9	70	F	Traumatic injuries	20-70	0-70	0-15	V. H.	1 hr.
10	52	M	Traumatic injuries	75	0-20	..	—	3 hrs.
11	Adult	F	Traumatic injuries	75	20	..	—	3 hrs.
12	Adult	M	Traumatic injuries	75	0-10	..	—	1 hr.
13	47	F	Traumatic injuries	65	50	..	—	24 hrs.
14	63	M	Traumatic injuries	65	0	..	—	10 hrs.
15	56	M	Traumatic injuries	80	55	0-2	—	24 hrs.
16	47	M	Pontine hemorrhage	75	..	..	—	8 hrs.
17	50	M	Cerebral hemorrhage	75	0-35	0-45	—	3 hrs.
18	Adult	M	Hypertension, chronic cardiac failure	75	0	0	—	3 yrs.
19	Adult	M	Cerebral hemorrhage	75	0-5	..	—	1 hr.
20	35	F	Traumatic injuries	75	10-50	..	—	10 hrs.
21	73	M	Traumatic injuries	10-70	0	..	—	12 hrs.
22	Adult	F	Traumatic injuries	0-60	0-1	0	—	1 hr.
23	65	M	Traumatic injuries	75	0-25	0-1	—	3 hrs.

V = Marrow of vertebra  
F = Marrow of femur  
H = Marrow of humerus

series of cases with and without lymphoid nodules in the marrow. It is obvious from the data in this table that there is no significant difference in the incidence of inflammatory lesions in the cases with and without lymphoid nodules in the marrow. This does not necessarily rule out association with inflammatory lesions since the nodules may be associated with certain lesions and not with others. Of the 265 cases in the first series, 78 or 29 per cent showed lymphoid nodules in the marrow.

Table II, which includes a brief diagnosis in each case, shows the data on the cases in the control series. Nine, or 39 per cent, showed lymphoid nodules in the marrow. Although the series is small, it may be stated that the nodules occurred frequently in the marrow where the incidence and extent of inflammatory lesions were reduced to a minimum. This is strong evidence against any association with inflammatory disease.

#### RELATION OF LYMPHOID NODULES IN THE BONE MARROW TO AGE

Data was available on the age in 230 cases in the first series of cases. Twenty-eight individuals were 40 years of age, or under, while 202 were older. Lymphoid nodules were present in the marrow in 3, or 10 per cent of the 28 individuals under 40 years of age, and in 64, or 32 per cent of the 202 individuals in the older group. The series of cases in the lower age group is small but when the figures are combined with those of Askanazy<sup>6</sup> and von Fischer<sup>7</sup> the results become significant. It is seen from Table III that in all series the nodules are more frequently found in individuals over 40 years of age than in those younger.

#### SUMMARY

In summary, it may be stated that lymphoid nodules occur frequently in the bone marrow and that they are definitely associated with active red marrow. They occur in a wide variety of diseases, are frequently found in cases where no infection or significant inflammatory lesion is demonstrable, and are also present frequently in the marrow in cases of sudden death from traumatic injuries. Local lesions in the bone marrow do not account for these foci. The lymphoid nodules are found more frequently in the marrow in individuals over 40 years of age than in that of adult but younger individuals.

TABLE III

*Relation of Lymphoid Nodules in the Bone Marrow to Age*

	Askanazy's series	von Fischer's series	Author's series	Total
Total number of cases examined under 40 yrs. of age	44	22	28	94
Number of cases under 40 yrs. of age with lymphoid nodules in marrow	10	10	3	23
Incidence of cases under 40 yrs. of age with lymphoid nodules in marrow	23%	45%	10%	24%
Total number of cases examined over 40 yrs. of age	82	39	202	323
Number of cases over 40 yrs. of age with lymphoid nodules in the marrow	33	28	64	125
Incidence of cases over 40 yrs. of age with lymphoid nodules in marrow	40%	72%	32%	39%

No pathological significance can be attached to the presence in the marrow of lymphoid nodules of the type, size and number described in this report. The evidence appears to justify the concept that the lymphoid nodules are essentially normal though perhaps variable constituents of the active red marrow of adult individuals.

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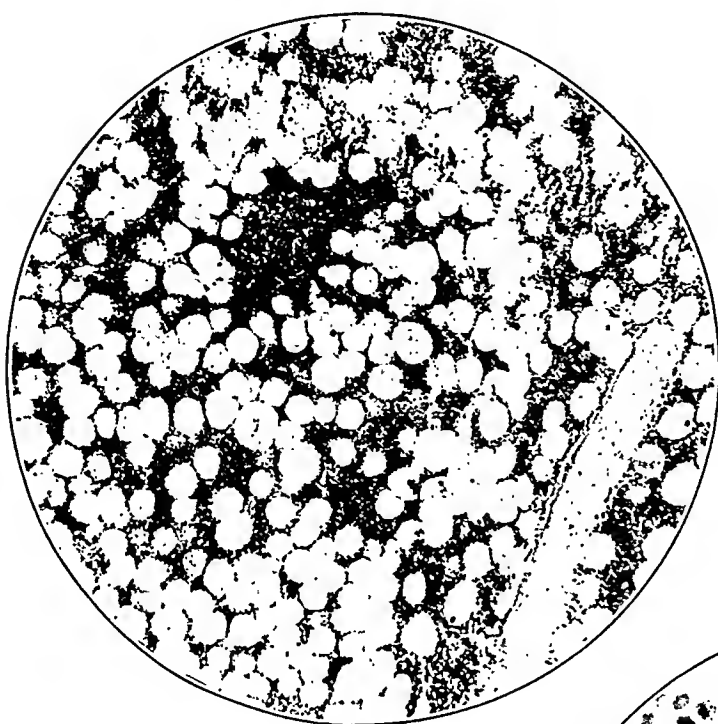
## DESCRIPTION OF PLATE

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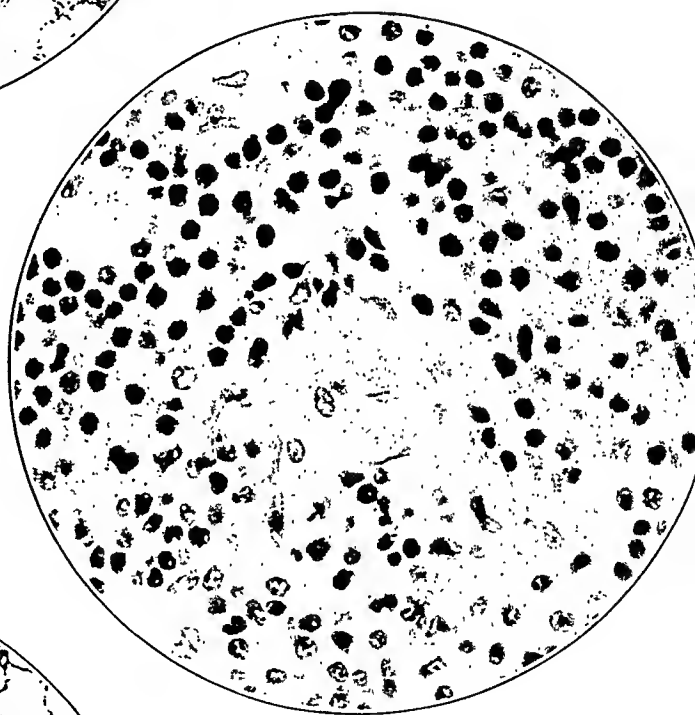
### PLATE 65

- FIG. 1. In the left upper quadrant there is seen a lymphoid nodule appearing as a unit structure in distinct contrast with the surrounding areas of granulopoiesis and erythroipoiesis. Hematoxylin-eosin stain.
- FIG. 2. Lymphoid nodule with a centrally placed hyalinized arteriole showing the type cell to be the small lymphocyte. Hematoxylin-eosin stain.
- FIG. 3. In the center of the field is seen a lymphoid nodule with a framework of reticular fibers. Foot's modification of the Bielschowsky stain.

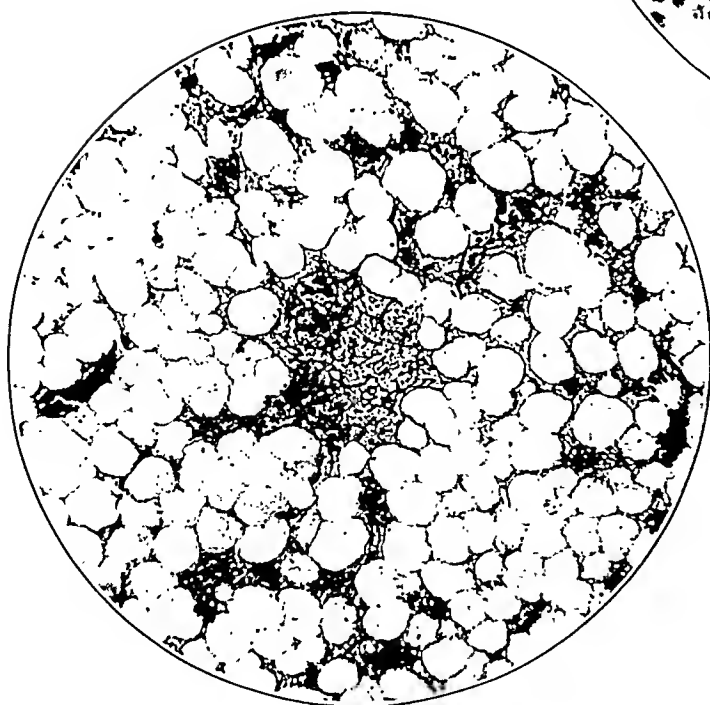




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## STUDIES ON A RAPIDLY DEVELOPING INTESTINAL ADENOMA IN A PIG \*

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In a previous publication 3 cases of intestinal adenoma in swine were described.<sup>1</sup> These appeared in herds among which severe intestinal disorders were common. The literature was reviewed and the various etiological concepts were discussed. Regenerative responses of the intestinal epithelium following severe destruction of the intestinal mucosa were also described. Unfortunately these field cases were not brought to our attention until the health of the animals was markedly impaired.

During the course of a nutrition experiment a ration consisting of 50 per cent skim milk powder, 48 per cent yellow corn, and 2 per cent minerals was fed to 1 of 4 groups of swine placed on dry lots. With one exception the pigs fed the above ration grew well throughout the experiment. Some of them were continued on this ration for 10 months. Pig No. 6117 gained in weight until about the 73rd day of the experiment when it reached the peak of its growth. At this time the animal was 105 days old (Chart 1). When weighed 11 days later it had lost 4 pounds, while its litter mate (No. 6118) had gained 20 pounds during the same period. When pig No. 6117 was weighed 6 days later it had lost an additional 4 pounds. There was a gain of only 2 pounds recorded in the next 11 days. One week later it was 8 pounds lighter (Chart 1). Four additional pigs, which were about 1 week older than pigs No. 6117 and No. 6118 when placed on this experiment, were included in this lot. The growth curve of these older pigs so closely resembled that of pig No. 6118 that they were not included in the chart.

About 2 weeks after pig No. 6117 ceased to gain in weight a mild progressive diarrhea was observed. Soon after the onset of the diarrhea small particles of yellowish gray croupous membrane were noted in the feces. The clinical appearance of the pig and the fecal findings were suggestive of a commonly observed form

\* Received for publication January 19, 1939.

of swine enteritis caused by *Salmonella suipestifer* as the primary factor, followed by the secondary invasion by *Actinomyces necrophorus*. The affected pig was removed from the experimental lot and the remaining animals were observed closely for the appearance of similar symptoms. The remaining animals of this lot and

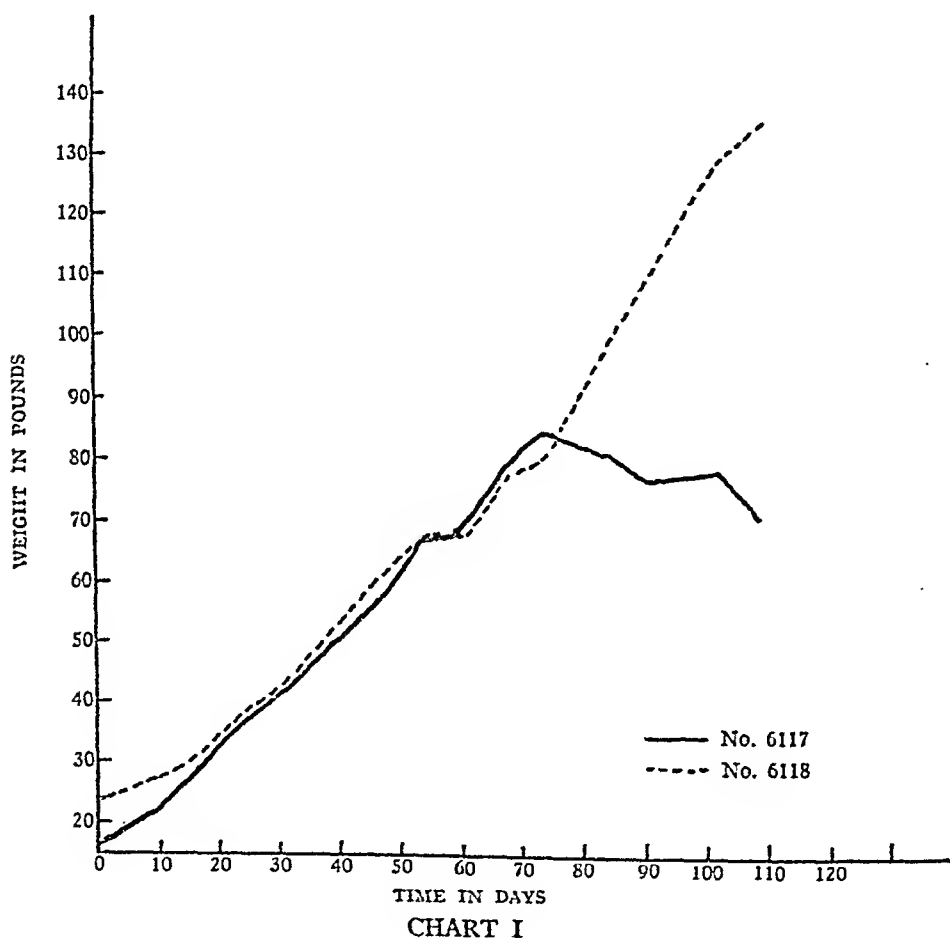


CHART I

of the adjoining lots remained well throughout the duration of the experiment.

Pig No. 6117 became quite weak and on the 108th day of the experiment, at which time the animal was 140 days old, it was destroyed for autopsy. In addition to the non-specific changes in the parenchymatous organs, the mesentery of the small and large intestines was greatly swollen and edematous. The terminal portion of the jejunum and the entire ileum showed numerous elevations on the mucosa. Some of the lesions were low, flattened convex newgrowths, while others were spherical. The ileocecal

valve, cecum and anterior half of the colon were involved in a similar manner. Most of the mucosal elevations were covered with a yellowish croupous membrane that was easily removed by a strong stream of water or by gentle rubbing. When these lesions and the intestinal wall were incised it was found that the newgrowths consisted of a proliferation of the mucosa which did not involve the submucosa. Only a few of the neoplastic formations showed traces of pedicle formation.

Histological study revealed an adenomatous proliferation of the intestinal epithelium. The cells were chiefly of the undifferentiated type, a few goblet cells being found at the outermost edges of the newgrowths. No indication of malignancy was found. Lateral branchings of the newly formed glands were not so well developed as in the previous cases cited.<sup>1</sup> In one section of ileum three acinal formations were found beneath the muscularis mucosa but the basement membrane was intact. The cecal sections of the adenomatous lesions revealed large numbers of *Balantidium coli* which were found in the croupous membrane and in the outermost parts of the proliferating epithelial tissue (Fig. 1). The protozoan forms did not show marked invasive power. The presence of so many *Balantidii* might raise the question of etiological relationship but it should be kept in mind that in swine this protozoan form does not show the same severe invasive power as that of the human. *B. coli* are not found in the small intestine of swine. That the same type of adenomatous formation in the jejunum and ileum failed to reveal *B. coli* offers additional evidence of a lack of etiological relationship of this protozoan form to adenomatosis (Fig. 2).

The growth curve of pig No. 6117 indicates that 30 days prior to the destruction of the pig the adenomatous process had developed sufficiently to impair the health of the animal. In all probability the process began before the pig was 100 days old.

The results of some analyses on the blood of pigs No. 6117 and No. 6118 are shown in Table I. The hematocrit value for pig No. 6118 is well within the normal range, while that of pig No. 6117 is definitely low. This appears to be the result of poor absorption and some loss by exudation, as evidenced by the extensive croupous membrane formation over the adenomatous tissue. In some microscopic fields varying numbers of red cells were present. There was no evidence of gross hemorrhage. The plasma

calcium was low in both pigs while the plasma magnesium for pig No. 6118 was the lowest in the group fed this ration. The high magnesium for pig No. 6117 does not appear to be related to the adenomatosis but more likely it is the result of some dietary factor. The phosphorous partition in the blood of pig No. 6118 was normal, while that of pig No. 6117 showed a high inorganic and a low lipid phosphorus. Pig No. 6117 was undoubtedly metabolizing its own body proteins. Normal pigs when fasted do

TABLE I  
*Blood Analyses*

	Pig No. 6117		Pig No. 6118	
	Whole blood	Plasma	Whole blood	Plasma
Hematocrit value	27		48	
	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.
Calcium		10.1		10.1
Magnesium	4.77	3.64	6.05	2.42
Chloride	280.0		270.0	
Inorganic phosphorus	19.3	14.9	11.01	8.73
Acid soluble phosphorus	26.0	15.2	51.0	9.7
Lipid phosphorus		3.3		4.8
Amino acid nitrogen	9.58	7.36	9.34	7.1

not show much change in the phosphorous partition. This is probably due to metabolism of fats from fat reserve. In the case of pig No. 6117 there was very little tissue fat. No significant impairment in nitrogen metabolism was indicated by the normal amino acid content of the blood of both pigs.

The relatively rapid and widespread growth of the adenoma at this early age and the fact that papillomatous growths of virus etiology are sometimes encountered on the tongue and in the oral cavity suggested the possible presence of an infectious agent. The affected intestinal tissue and tumors of pig No. 6117 were rinsed with distilled water and one portion was preserved in 50 per cent sterile glycerin overnight, while the remainder was placed in saline in a refrigerator. Ten cc. of a Berkefeld N filtrate was injected intraperitoneally in a 5 months old pig. Material from the same filtrate was placed on the tongue by means of a hypodermic syringe and needle. At the same time the mucous membrane of the tongue was scarified. Additional filtrate was injected into

the submucosa of the tongue and into the cutis and subcutis of the chest region. About 250 cc. of filtrate was given by stomach tube to a 2nd pig. A 3rd pig of the same age was fed on 2 successive days finely cut adenomatous tissue that had been stored in 50 per cent glycerin overnight. A 4th pig was fed finely cut tissue from the adenomatous intestine which had been collected and held in saline solution. When the 4 pigs were subsequently destroyed no evidence of adenomatous growth could be demonstrated.

Bacteriological examination of the tissues of pig No. 6117 failed to demonstrate the presence of *S. suispestifer*. The failure of the pigs to develop enteritis when fed unfiltered intestinal tissue from pig No. 6117 constitutes further evidence of the absence of an infectious form of enteritis.

### SUMMARY

A case of rapidly developing intestinal adenomatosis in the pig is described. The process did not appear to be associated with an infectious agent. The low grade invasive power of *B. coli* in the pig and its absence in the lesions of the small intestine indicate a lack of correlation with the newgrowth.

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## DESCRIPTION OF PLATE

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### PLATE 66

FIG. 1. Section of adenomatous tissue from the cecum with croupous membrane containing *B. coli*.

FIG. 2. Section from the ileum showing the adenomatous growth with croupous membrane formation. Red blood cells are present in the depths of the exudate. No *B. coli* are present.





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## CYTOLOGICAL CHANGES INDUCED IN THE HYPOPHYSIS BY THE PROLONGED ADMINISTRATION OF PITUITARY EXTRACT \*

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When Harvey Cushing first described the syndrome that now bears his name he promptly appreciated the fact that pituitary basophilism, as was true of acromegaly (*i.e.* pituitary acidophilism), would be better understood if it could be experimentally reproduced. An attempt to achieve this result by the injection into a puppy of a crude pituitary gonadotropic extract (presumably of basophilic elements) was described in 1934 by Thompson and Cushing.<sup>30</sup> While the animal developed a condition bearing certain resemblances to the clinical syndrome, it showed at the same time effects more or less characteristic of pituitary deficiency, namely, inactivation of the thyroid, adrenal cortex and gonads, and a failure to grow.

Equally striking results obtained in attempts to reproduce the syndrome in other animals subsequently led one of us (K. W. T.) to make a correlated study of the antihormones, discovered by Collip and his co-workers,<sup>1, 3, 4</sup> in order to interpret the rôles these substances may have played in the injected animals. These

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studies established the non-species-specific quality of the antihormones,<sup>15, 27, 31</sup> and accounted for the observed inactivation of the animals' own pituitary hormones.

The evidence now available from several sources (Du Shane *et al.*,<sup>6</sup> Gordon *et al.*,<sup>7</sup> Rowlands and Parkes,<sup>15, 16</sup> and Twombly<sup>32</sup>) suggests that the antihormones are antibodies which are formed in the injected animal in response to an antigen to which is linked the pituitary hormone. The antihormones are readily produced when crude pituitary extracts from a foreign species are injected into an animal, but not when these extracts are sufficiently purified or the antigenic complex is separated from the hormone (Werner,<sup>35, 36, 37</sup> confirmed by Thompson, unpublished experiments). These antisubstances have not usually developed when the test animal has been injected with extracts derived from the same species (sheep pituitaries in sheep, Rowlands,<sup>14</sup> Thompson,<sup>26</sup> Collip<sup>3\*</sup>; rat pituitaries in rats, Smith<sup>24, 25</sup>; and prolactin in man, Twombly<sup>32</sup>). When first developed in the animal, the antihormone is ordinarily specific only for the extract injected, but after prolonged injections under suitable conditions the antihormones become non-species-specific; in other words, they inactivate the like hormones from many species of animals including those of the injected animal itself (unpublished data, Thompson).

Definite physiological effects are caused by the antihormones in animals. The gonadotropic antihormone, for example, under suitable conditions causes: (1) an inactivation in test rats of gonadotropic hormones from several species of animals, including man<sup>15, 30</sup>; (2) the failure of the sexual maturity of young rats<sup>30</sup>; and (3) the abortion of mice,<sup>30</sup> rats,<sup>30</sup> rabbits<sup>11, 30</sup> and dogs<sup>29</sup> (the latter 2 species may thus be aborted at any stage of pregnancy). Anderson and Collip have reported the lowering of the basal metabolic rate of animals treated with thyrotropic antihormone.<sup>1, 4</sup>

While these antihormone studies were in progress, clinical case reports of the so-called Cushing syndrome from various sources made it clear that a condition almost indistinguishable from that accompanying a basophilic adenoma may occur with an adenoma or carcinoma of the adrenal cortex,<sup>2, 8</sup> an oat-cell tumor or carci-

\* Collip observed weak antihormones in the serum of 2 out of 4 sheep that had been injected with sheep extract. It is conceivable that denaturation of the extract may have been responsible for the results.

noma of the thymus,<sup>9</sup> and in a few instances with no demonstrable adenoma of any organ (unreported cases). Furthermore, Crooke<sup>5</sup> and later Rasmussen<sup>12</sup> noted a characteristic cytoplasmic hyalinization in the basophilic cells of the pituitary in verified cases with the syndrome. This change is found in most of the basophilic cells of the anterior lobe, but it does not occur in the cells of the basophilic adenoma itself, should one be present. These specific changes have been observed only in the glands removed from human cases and heretofore have not been described in experimental animals. Crooke claimed this basophilic hyalinization to be the only single "pathological common denominator," possibly the essential lesion, of the syndrome.

An analysis of the data concerned with the Cushing syndrome made it appear more than likely that in the antihormone experiments some associated changes in the anterior pituitary glands of the animals might also be expected, and certainly should be sought.

The present report concerns itself with a study of the cytological changes in the pituitary glands of three groups of animals (A, B and C), some of which had been subjects for the attempts to reproduce the clinical syndrome of basophilism, and others of which were subjects for the investigation of the antihormones.

The pituitaries first studied were those from 2 dogs (A) that had received prolonged injections of a crude sheep pituitary extract. Marked cytological changes appeared in these glands. It was impossible to say whether the changes in these two hypophyses were due directly to the injected extract or to the physiological action of the antihormones which were extremely active in the serums of these animals. There are, to the authors' knowledge, no cytological data to indicate what effect, if any, the antihormones have upon the anterior pituitary gland. To this end 2 other dogs (B) were injected with a suitable antihormone serum, in order that their hypophyses might be studied. The third pair of glands to be studied were the hypophyses of 2 sheep (C) which did not develop antihormones during a prolonged course of injections of the sheep extract. Before proceeding to a cytological description of the hypophyses of these animals, it seems advisable to state briefly the experimental observations.

## THE ANIMALS

*(A) The Dogs Injected with Sheep Pituitary Extract*

*Dog No. 1:* A female fox terrier puppy was injected daily for 4 months with 25 cc. of an extract of sheep pituitary glands. This extract, which had been used in many of the antihormone experiments of one of the authors (K. W. T.), was prepared by alcohol precipitation after a method described by van Dyke and Wallen-Lawrence.<sup>33</sup> A marked atrophy of the thyroid, adrenals and gonads was noted and reported in the aforementioned publication (Thompson and Cushing<sup>30</sup>). The grossly normal pituitary gland was preserved for future study. The antihormones were not investigated in this animal, but later observations of other puppies similarly treated have indicated that the effects observed may be attributed largely to these very active antisubstances.

*Dog No. 2:* An adult female shepherd dog was injected daily for 210 days with 25 cc. of an extract identical with that given to dog No. 1. The animal at first developed in her serum the augmentary principle (Thompson<sup>28</sup>), and she later developed, in succession, antihormones for the gonadotropic hormone of sheep pituitary extract, pregnant mare serum, and human pituitary glands. During the period of the injections this animal's fur became coarse and sparse, as if she had been thyroidectomized, and she developed hypercholesterolemia. At autopsy the thyroid, adrenals and gonads were found to be atrophied. The thyroid epithelium, fixed in Zenker's plus acetic acid, was flattened, and the colloid had no absorption vacuoles.

*(B) The Dogs Injected with Canine Antihormone*

*Dog No. 3:* An adult female mongrel dog was injected subcutaneously daily for a period of 32 days with 10 cc. of canine antihormone serum. The donor was a Collie dog that had been injected daily for 3 years with the above mentioned sheep pituitary extract. This particular serum inactivated the gonadotropic hormones of many species, and it invariably produced abortion of pregnant dogs. In addition to the antihormones the serum contained considerable amounts of the antidiuretic principle, which

also was present in the sheep extract. The serum did not contain a measurable amount of the oxytotic principle.\*

At autopsy the pituitary gland of the dog injected with this serum was grossly normal, but the thyroid, adrenals and gonads appeared smaller than normal. Histologically the thyroid showed all the signs of subnormal functional activity. This animal did not develop hypercholesterolemia.

*Dog No. 4:* An adult female mongrel dog was injected daily for 30 days with 10 cc. of the same antiserum. The serum in this case was injected intramuscularly on one day and intravenously the next day because the intravenous route of administration was found to be more satisfactory for the induction of abortion of pregnant dogs. At autopsy the thyroid, adrenals and gonads appeared inactive. Histologically the thyroid was slightly more active than that of dog No. 3, but it was no more active than normal. This animal also failed to show hypercholesterolemia.

### *(C) The Sheep Injected with Sheep Pituitary Extract*

*Ewe No. 1:* An immature ewe was injected daily, beginning at the age of 4 months, with 25 cc. of the same sheep pituitary extract that was given to dogs Nos. 1 and 2. During the subsequent 6 months this ewe's serum was tested at intervals and was found to contain no gonadotropic antihormone. Before the injections were started, however, the serum contained a principle which inactivated thyrotropic hormone. During the period of injections the genitalia and nipples continually showed signs of stimulation, and at autopsy, after 6 months of injections, the internal genitalia were found to be considerably hypertrophied. As compared to a half-sister control of the same age, the adrenals and thyroid were approximately normal. Histologically the thyroid showed a normal degree of activity. The hypophysis was normal in size.

*Ewe No. 2:* This ewe, a twin of ewe No. 1, was subjected to similar treatment except that the extract injected was prepared by alkaline extraction of an acetone-dried powder of sheep pituitary glands. This animal did not develop gonadotropic antihor-

\* For the tests of the posterior lobe principles, the authors are indebted to Dr. Alfred Z. Gilman of the Laboratory of Pharmacology, Yale University Medical School, New Haven, Conn.

mones, and the autopsy revealed the same effects of the injections as were found in ewe No. 1.

*Ewe No. 3:* The control lamb was a half-sister of ewes Nos. 1 and 2. Their parents were from a highly inbred stock. The control was fed approximately the same diet and was 10 months old when autopsied. Her serum contained no gonadotropic antihormone, but, like the other 2 lambs, contained an antithyrotropic principle. Her gonads and uterus were not hypertrophied.

### THE CYTOLOGY OF THE PITUITARY GLAND

*Technical Methods:* The pituitary gland of dog No. 1 was removed 2 hours after death and fixed in neutral 10 per cent formalin. All other hypophyses were removed promptly after the death of the animal by air embolism and immediately fixed in Zenker-formalin.

A brief outline of the technic used to prepare the tissues for microscopic examination is given below:

1. After 8-24 hours fixation, tissues are washed in running water for 15 hours and then run through graded alcohols to 95 per cent alcohol where they remain for 5-12 hours, depending on the size of the tissue block. The tissue is then placed in absolute alcohol to which is added an equal volume of ether. After 2 hours, an equal volume of 2 per cent celloidin is added. The tissue then remains in this mixture for 5-18 hours.

2. Tissue is run through celloidin as follows: 48 hours in 2 per cent, 48 hours in 4 per cent, and 48 hours in 6 per cent. The tissue is then cast in 6 per cent celloidin in paper boxes and is hardened overnight in chloroform vapor in an air-tight jar. Next, trim the celloidin as close to the tissue as possible and place the block in carbon disulphide for 24 hours or longer if the block continues to float.

3. Place the block in carbon disulphide-paraffin mash (equal parts 62° C. paraffin and carbon disulphide) for 5-7 days in a place just sufficiently warm to keep the paraffin melted. Carry the block through 1 change of melted 62° C. paraffin for from 5-25 minutes and cast in freshly filtered 62° C. paraffin which has been heated to about 70° C. Immerse in warm water (not over 45° C.) for hardening.

4. If the tissues are brittle or difficult to cut, shave the block



until a small portion of the tissue is exposed and submerge in water for from 2 to 5 days before sectioning. Ice, or ice water, is usually necessary to keep the knife and the block cold during cutting at 2 or 3  $\mu$ .

5. The following mixture is used for mounting and spreading the sections (after egg albumin has been applied to the slide). To 10 cc. of acetone add 5 drops of methylbenzoate and mix well; add 40 cc. of distilled water. In spreading the sections, the best results are obtained by rapid cautious use of a hot plate at a temperature 5–10 degrees higher than the melting point of the paraffin used.

*Staining:* Sections are run through xylol and absolute alcohol into a solution of 3 parts of oil of cloves and 1 part of absolute alcohol for 10 minutes. Proceed through graded alcohols to distilled water. Flood the slides with Altmann's 20 per cent acid fuchsin solution and gently heat to steaming (with an alcohol lamp). Allow 5 minutes for cooling. Differentiate, if necessary, in picric acid alcohol as recommended in the Altmann method (1 part of saturated alcoholic picric acid and 7 parts of 20 per cent alcohol).

Wash the slides carefully in distilled water and place in 1 per cent phosphomolybdic acid for from 1 to 2 hours. Place the slides directly (do not rinse) into aniline blue as prepared by Masson for about 1 hour (longer or shorter time as required). Wash the slides in distilled water and shake off the excess water. Rinse quickly in 95 per cent alcohol and absolute alcohol, clear in xylol and mount.

If desired, hematoxylin may be successfully used as a nuclear stain. It should precede the acid fuchsin.

*(A) Anterior Hypophyses of Dogs Injected for an Extended Period with Sheep Pituitary Extract*

The hypophyses of dogs Nos. 1 and 2 are profoundly altered. Although essentially similar, the cytological changes are more pronounced in dog No. 2 and may be attributed to the much longer period of injection. The basophils are much larger than normal. In some the distinct cytoplasmic granulation, characteristic of the basophils in dogs, is still present. In many cells, however, the character of the granulation has changed. Granules are frequently

gathered into spherical masses of irregular size, as shown in Figures 1-6. While such cells may be found by search in the normal hypophyses, there is no question of their great numerical increase in these injected dogs.

Most of the basophils show extensive vacuolation. The vacuoles are of three types. In the first type they appear as clear spaces, suggesting that they contain a non-stainable substance, or that the original substance has been technically removed (Figs. 19-24). A second group of vacuoles is filled with a substance staining a clear pale blue. These vacuoles in early stages are small and scattered throughout the cytoplasm. In other cells the vacuoles have coalesced to form more extensive vacuolar inclusions. A third type, perhaps the most common, has a deeply basophilic amorphous substance. Here again, many cells will show small vacuoles distributed among the coarse masses of basophilic granules (Figs. 7-17). In later stages the vacuoles have expanded and united to occupy large portions of the cell, or have replaced the granular cytoplasm almost entirely. Figures 11 and 12 show that the large vacuoles are formed by coalescence of the smaller ones.

In this widespread disturbance of the basophils it is readily possible to demonstrate cells in which the vacuolation is identical with that in the typical castration cell of the rat or monkey (Fig. 17). Other basophils contain the more extensive irregular vacuolar distortion regularly seen in the hypophysis of the thyroidectomized rat (Fig. 22). Still other cells show a combination of granular and liquefied areas which are indistinguishable from the hyalinization of basophils described by Crooke in the Cushing syndrome of pituitary basophilism (Fig. 31).

The fact is evident that regardless of their final configurations, the types of vacuolation begin in a similar manner, essentially a liquefaction of the cytoplasmic granules to give a basophilic amorphous substance, perhaps better called colloid-like than hyaline. Previous careful cytological analysis of the onset of basophilic changes which follow castration and thyroidectomy have shown that the early stages of vacuolation are indistinguishable. Experimental evidence supports the contention that these vacuolations are inseparable for it is possible to prevent or remove vacuolation after thyroidectomy by administration of estrone in doses still within the physiological range (2-5 r. u. per day)

(Severinghaus,<sup>22</sup> and Nelson and Hickman<sup>10</sup>). Castration changes, conversely, have not been cleared up by the administration of thyroxin. One might expect that this would be difficult to accomplish, for strangely enough basophilic vacuolation occurs in both hypo- and hyperthyroid conditions (Severinghaus *et al.*<sup>18, 19</sup>).

The study of the hypophyses of these massively injected dogs points still more clearly to the conclusion that basophilic vacuolation is a characteristic retrogressive alteration which the cells undergo when their normal physiology is disturbed. A previous suggestion that the Cushing syndrome is one of these disturbances now seems even more justified, and we are inclined to regard the Crooke changes as an aspect of the general granule liquefaction which also appears after castration or thyroidectomy.

Furthermore, a study of these glands again confirms one in the opinion that all of these basophilic changes are atrophic in character. This in no sense implies that the destruction of cytoplasmic granulation through liquefaction produces a substance which is hormonally impotent. There is, however, no good experimental evidence to indicate that the widespread vacuolation due to either castration or thyroidectomy is associated with any increase in the respective gonadotropic or thyrotropic hormone content of the anterior hypophysis. The increase of gonadotropic hormone which occurs after castration is much more logically related to the great increase of large granular basophils, while after thyroidectomy, if any increase in the thyrotropic hormone content occurs, which is very questionable, the same correlation is indicated.

Sizeable areas in the hypophyses of these dogs are composed of secretory active basophils. These cells cannot be identified by reference to the Golgi apparatus, for the Golgi region differences between the two types of chromophils in the dog's hypophysis are not clear-cut.<sup>17</sup> The cells, however, have the general characteristics of basophils. They are much larger than acidophils (Fig. 25). The cytoplasmic granulation is fine and stains a light slate blue color rather than the dark blue of the typical basophil. The mitochondria, though faultily preserved with Zenker-formalin, are numerous and the Golgi region is hypertrophied. These cytoplasmic features indicate a metabolically active cell, producing and liberating a secretory product at a rate above normal. The cells seem to have developed from the chromophobes which are

now entirely missing from such regions. It is not impossible that these basophils which are now in a phase of hyperactivity will later succumb to atrophic vacuolar changes.

There is some clinical evidence to indicate that patients with the Cushing syndrome pass through a period of hyperthyroidism which is followed by hypothyroid symptoms. This reversal of thyroid activity would produce correlated anterior lobe changes.

The changes which the acidophils of the injected dogs undergo are much less spectacular, but nevertheless real. If the proportion of acidophilic cells differs from the normal, it could be established only by statistical methods on a much larger series of animals. Suffice it to say that acidophils are abundant. From the fact that basophilic changes in these glands resemble in part those which follow thyroidectomy, the reader may have expected a great diminution or even an absence of acidophils. However, thyroidectomy in the dog does not result in the striking disappearance of acidophils which one sees, for example, in the anterior hypophysis of the rat (unpublished data, Severinghaus).

Acidophilic regions of the gland do not present a uniform appearance. In certain areas the cells are small and irregularly shrunken, with varying degrees of granulation. They have pyknotic nuclei which stain deeply basophilic (Fig. 27). Scattered among these acidophils are many chromophobic cells with exactly the same nuclear characteristics. These two types of cells are typical of stages in the reversion of acidophils to chromophobes in the cycle of secretion previously described in the human hypophysis.<sup>21, 23</sup> Such acidophils will revert to chromophobes but after a nuclear reorganization may at any time begin a new cycle of acidophilic activity. In this connection it is interesting to record that large areas of normal acidophils and chromophobes are also characteristic of the anterior lobe of these injected dogs (Fig. 26).

Finally, it may be pointed out that whereas the dog's anterior pituitary gland contains many small basophilic colloid bodies, they seem decidedly increased in these experimental animals. These masses are frequently surrounded by a row of cells so that they occupy the center of a cord of cells. At other times they appear along the connective tissue framework of the gland and are therefore between the cell cords. Ciliated cells have been seen on occasions to border upon larger colloid inclusions, but these

seem different in character from those described above. The origin and nature of the colloid remains obscure.

(B) *Anterior Hypophyses of Dogs Injected with Canine Antihormone*

A single glance at the anterior hypophyses of these dogs reveals that they have undergone striking alterations. Four features are especially striking: (1) the scarcity of typical areas of chromophobic cells; (2) the replacement of normal basophils by hyalinized or vacuolated cells, or by large sparsely granulated cells rich in mitochondria; (3) modifications in the acidophils; and (4) a marked hyperemia and edema of the gland.

The absence of the usual large number of chromophobes gives the anterior lobe a highly granular appearance. Chromophobes have not entirely disappeared, but they are greatly reduced and scattered individually throughout the glandular stroma, in contrast with the grouped arrangement of the normal gland. This unquestionably adds to the impression of their scarcity (Fig. 32).

The decrease in chromophobes is directly correlated with a marked numerical increase of large granulated cells of basophilic character. The coloration of these cells is extremely variable. It varies from the occasional normal dark blue of the normal basophil through varying shades of purple with an increasing reddish cast. Granulation is progressively coarser and more scattered in such a series of cells. There is little reason to question the basophilic lineage of any of these cells. The changes in coloration are due to varying degrees of degranulation of the specific basophil granules and a simultaneous increase in the mitochondrial content of the cytoplasm. With Zenker-formalin fixation the mitochondria have been preserved, although less perfectly than with the chromosmic fixation.

The staining of alternate  $3\ \mu$  sections with copper hematoxylin clearly demarcates the acidophils and shows the varying degree of mitochondrial development in the basophils, since in the latter cells only the mitochondria of the cytoplasm stain. The cytoplasm of the dark basophils appears clear with this technic. In the purplish cells the scattered mitochondria granules stand out sharply against the clear cytoplasm. They have a coarse irregular aspect and are in no way suggestive of acidophilic granules.

The frequent presence of a large Golgi zone as well as the mitochondrial increase leads us to believe that these cells are active both in the production and in the release of a secretory substance. Their rapid increase in large numbers at the expense of the chromophobes indicates that there are many undifferentiated chromophobes which have basophilic potentiality or that chromophobes of the acidophil line are able to return to an undifferentiated state and then give rise to basophils. In the dog one is not able to differentiate the granular cells with accuracy by the shape and position of the Golgi apparatus, so that any attempt to analyze the chromophobes, as can easily be done in the rat,<sup>17</sup> is impracticable.

Many of the mature basophils show evidence of the onset of vacuolation as described in the previous section of this paper. An irregular and massive clumping of the specific cytoplasmic granules has occurred in most of the cells. The deep blue granular masses are sometimes distributed at random throughout the cell, but more frequently are concentrated centrally near the nucleus. Surrounding them is a non-granular, amorphous cytoplasm which exhibits a variable affinity for the dyes. In dog No. 4 the hyaline substance of the majority of these cells stains a pinkish slate color even after over-extraction of the acid fuchsin and the forcing of the aniline blue. The similarity of these cells to the hyalinized basophils of the human anterior lobe in cases of pituitary basophilism is most striking (Fig. 31).

The acidophils are present in such numbers as to suggest no numerical deviation from the normal. Many of the cells are small and compact and well granulated, but some are large and have a prominent Golgi zone. In many of these the granulation is more sparse. The nuclei are on the whole normal, there being no excessive amount of lobulation or pyknosis. Scattered among the normal acidophils are cells which stand out because of their brilliant fuchsin staining. These cells range in size from cells smaller than the normal acidophils to cells considerably larger. Their shape is often irregular. Cytoplasmic granules cannot be resolved. The nucleus is pyknotic and basophilic. These cells, plentiful in the antihormone serum-injected dogs, can be found by search in the dogs injected with pituitary extract for prolonged periods. Their numerical difference in the two groups is obvious, even with

a cursory examination of the slides. These atypical acidophils are not unlike cells to be described presently in the anterior lobe of the pituitary in sheep following prolonged injections of pituitary extract. In the sheep one can observe stages in the transformation of normal granular acidophils into these cells. In the dog one has no clue either as to their origin or significance.

The hypophyses of the antihormone serum-injected dogs show a marked hyperemia and edema. A careful microscopic study of these conditions has revealed some very interesting facts. The capillaries appear either as large sinusoids distended with blood cells, or as contracted capillaries of small diameter. These latter usually run through an edematous area which approximates the size of the most distended capillaries (Figs. 32, 33). In general, the edematous area is filled with a blue granular substance which has all the characteristics of basophilic granules. In fact, under the lower magnifications, one gains the impression that these extracellular granules are areas of basophilic cells. On numerous occasions the granules of such areas are directly continuous with the dispersing basophilic granules of bordering cells. This phenomenon can also be found at places along the distended capillaries (Fig. 30). It is common also to find blood cells within the edematous area outside the capillary wall. Since the tissue is excellently preserved, there can be but one interpretation, namely that the capillaries are in frequent communication with the edematous areas and that active degranulation of the cells is taking place into these areas as well as into the capillaries directly. The cytoplasmic granules are still recognizable in the blood plasma \* (see Figs. 30 and 33).

*(C) Anterior Lobe of the Ewe Lamb after Prolonged Injection  
with Sheep Pituitary Extract*

The hypophyses to be described in this section are from 3 highly inbred ewe lambs 10 months of age. The 2 injected animals were twins, and the normal control a half-sister.

\* For the sake of emphasis one of us (A. E. S.) wishes to state that during the last 10 years devoted largely to a cytological study of the endocrine glands, granular substances within the blood plasma have been noted on numerous occasions. On no occasion, however, have these been looked upon as cytoplasmic in origin, but rather as coagulation products of the plasma. In the present highly activated glands, however, we have not the slightest reservation concerning the identity of these granules.

The anterior lobe of the pituitary in sheep is normally predominantly acidophilic. Limited areas of basophils are found peripherally and may extend here and there as solid cords of basophilic cells into the deeper portions of the gland. In addition to these cords, there are scattered isolated basophils throughout the entire glandular area. As in all other species, the basophils may be found in varying degrees of degranulation but the majority of the cells have a cytoplasm well filled with distinct basophilic granules which stain brilliantly with aniline blue after Zenker-formalin fixation. The Golgi region is not sharply demarcated in the heavily granulated cells, but in the degranulating cells it is seen as a prominent cytoplasmic structure, somewhat acidophilic in coloration due to the abundance of mitochondria in this region.

The acidophils have a distinctly granular cytoplasm and a nucleus with a network of chromatin and one or two large nucleoli.

However, various modifications of the acidophils occur, and these again closely approximate the stages described for the secretory cycle in the acidophils of the human hypophysis (Severinghaus<sup>21,23</sup>). In addition to nuclear and cytoplasmic granular changes, the acidophils of the sheep exhibit a great variety of shapes. Round, ovoid or polyhedral shapes are common. Frequently cells are elongated and it is not uncommon to find a whole row of elongate, almost columnar cells bordering a sinusoid. In a recent paper Warbritton and McKenzie<sup>34</sup> describe as many as nine types of cells in the ewe, in place of the traditional three. Among their criteria of classification are the shape of cells and the degree of granulation. We have found no cells in the anterior lobe of the pituitary in sheep which could not be recognized as either acidophil, basophil or chromophobe. Variability in the size and shape of glandular cells, which constantly occurs with the elaboration and discharge of secretory products by the cell, can hardly be acceptable criteria for separating cells into distinct types, in the sense that chromophobes, acidophils and basophils are separable.

One modification of the acidophil is deserving of special mention. Occasional cells are present with a homogeneous, non-granular cytoplasm which stains brilliantly with acid fuchsin. The nuclei of these cells are highly pyknotic and basophilic. The cells



are very irregular in shape. It is evident from cells in which granular and non-granular areas are associated in varying proportions that these hyalinized cells are modified acidophils.

The hypophyses of the twin ewes which were injected with an extract of sheep pituitary gland are clearly modified. The most striking change is an almost universal degranulation of the basophilic cells (Fig. 28). It requires considerable search to locate a normally granulated basophil. Mitochondria and the Golgi apparatus are prominent in the degranulating basophils. The acidophils seem increased in size, are compactly granulated, and stain more brilliantly than do the cells of the control. The cells with a brilliant acidophilic hyaline cytoplasm are much more numerous, as are the transitional stages in which a partial granulation still remains. Areas of small acidophils with pyknotic basophilic nuclei are common. These glands give cytological evidence in both chromophilic cells, but especially in the basophils, that the secretory activity of the anterior hypophysis is considerably increased over the normal.

#### DISCUSSION AND CONCLUSIONS

We are not able at this time to offer a thorough interpretation of the observations that have been described above. We may, however, emphasize what seem to us to be the more important observations. In the first place, the "Crooke changes" heretofore described only in the human pituitary gland have now been experimentally produced in dogs. Although individual cells with the Crooke change were found in the hypophyses of the dogs that had prolonged injections of anterior pituitary lobe extract, they were much more common in those dogs injected with the antihormone serum. Basophilic changes, characteristic of castration and thyroidectomy, on the contrary, were the outstanding characteristics of the former group presumably because of the longer period of injections.

The profound changes in the anterior lobe of the pituitary described in these experiments would have little interest or value unless we attempted to gain from them some insight into the physiological processes with which they are associated. In other words, the question of major importance is, "How are these cytological changes produced?" It is obvious that the proper correla-

tion of sufficient data of this character must eventually lead to a correct understanding of the glandular functions.

A number of possibilities immediately suggest themselves. In the first place, it is conceivable that the anterior lobe is being damaged by cytotoxins which the extended injections may call forth. Or again, the changes may be produced by the direct effects of the injected pituitary extract or indirectly through the increased secretions of other endocrine glands which the injections may have activated. In this connection it is necessary to know whether the effective principles are of anterior lobe origin or whether other pituitary hormones (antidiuretic, and so on), which we know to be present in the extract, are also involved. In the third place, the anterior lobe changes may be due directly or indirectly to the antihormones which have been elaborated. Finally, one must ask if the changes indicate the elaboration of the antihormone by the hypophysis itself.

Some of these questions seem rather easily disposed of. It is not likely that the antidiuretic principle is responsible for the gross cytological changes in the anterior lobes of these dogs. The hypophysis of the ewe injected with the sheep extract known to contain the antidiuretic hormone gave evidence of secretion activation but showed none of the profound basophilic changes seen in the dogs injected with the antiserum or the pituitary gland extract.

The evidence further indicates that the observed changes are not to be attributed to the actual elaboration of the antihormones by the pituitary glands. Considerable data are now available to show that the antihormones are produced in the body tissues even in the absence of the hypophysis. Our results contain nothing to lead us to question the assumption that the antihormones are produced in such a likely site, for example, as the reticuloendothelial system.

Direct experimental evidence seems difficult to obtain either for or against the supposition that damage to the hypophysis by the action of cytotoxins may result from prolonged injection of pituitary extract. The possible rôle of the Forssman reaction in this problem remains to be investigated.

The dissimilarity of the effect upon the dog and sheep hypophysis and upon other endocrine glands after similar prolonged

injections of pituitary extract makes it difficult to assume that the anterior lobe changes are due solely to the direct action of the injections. This fact likewise lessens the possibility that the injections activated the other endocrine glands which in turn produced the changes finally seen in the hypophyses. It is true that the initial effect upon the hypophyses in all of the animals here described has seemed to us to be a stimulation of secretory activity. We believe this to be due to an activation, by the injections, of the gonads and thyroid (adrenals?) whose hormones in turn affect the hypophysis. The anterior hypophysis of the injected ewe remains in this state of hyperactivity even after 6 months of daily injection. The antiserum-injected dogs have hypophyses which show in part signs of increased activity and in part evidences of retrogression, perhaps the result of a preceding exhaustion through abnormal activity. The pituitary-injected dogs alone show the widespread changes, especially in the basophilic cells, which combine the characteristics of the Cushing syndrome of castration and of thyroidectomy.

The early stages in the phenomena of basophilic vacuolation, which we regard as an indication of reduced rather than increased secretory activity, might be expected in the hypophyses of dogs injected with the antihormone serum. If the antihormones begin a neutralization of the hypophyseal hormones, as in the phenomena of immunization, then the thyroid, gonads and other glands would begin to suffer the effects of pituitary deprivation. The result of their progressively decreasing activity should eventually be an effect upon the anterior lobe simulating a total ablation of this organ.

Those anterior lobe features resulting from antihormone-serum injections, which we have interpreted above as evidence of increased hypophyseal secretion production and release, are more difficult to understand. We know that the earliest effects upon the hypophysis of total ablation of the thyroid or gonads are in part activating rather than depressing. This is shown by the great numerical increase of basophils at the expense of chromophobes. Cytologically, however, such cells proceed rapidly to vacuolation and do not give evidence of increased secretory release as do those large areas of basophils in the hypophyses of antihormone-serum-injected dogs. Moreover, we have no right to assume that by de-

pressing a gonad or thyroid we immediately completely inactivate it, and thus produce in the experimental animal a situation comparable to the earliest period of total glandular ablation.

It should be pointed out that many investigators believe in the activation of secretory processes in the pituitary gland by a suppression of the gonads. This view is based on a considerable body of evidence, especially that derived from certain parabiotic experiments and from a comparative analysis of the hormone content, supposedly of anterior lobe origin, in the urine of pre- and post-menopausal women. The evidence for a diametrically opposite view, which cytological studies in a wide variety of experimental procedures have confirmed without exception, has been presented elsewhere (Severinghaus<sup>22</sup>). Although progress is evident of a harmonization of these conflicting views, the matter is far from being settled. We need not be unduly exercised over these discrepancies, but that we recognize them is of first importance. Discrepancies are the rich deposits of discovery. They indicate the points at which our efforts should be redoubled.

One great difficulty in attempting to interpret the results of the present experiments lies in the fact that massive dosages have been employed. Errors may readily be committed by attempting to impute the normal physiological glandular interplay to experiments in which the whole organism may have been thrown into a state of physiological disorder by an intolerable, massive administration of a certain potent hormone. In this condition it is conceivable that the activity of a gland may be altered quantitatively and qualitatively, and that further complications arise if, at the same time, the end organs lose their capacity to respond normally.

Fully realizing the difficulties involved, we venture to go beyond a mere recital of our cytological findings and state that to us the most reasonable interpretation of the cytological changes in the anterior hypophysis, due to prolonged administration of pituitary extract, is obtained by assuming that the following reactions occur in sequence in the experimental animal:

1. Injection of pituitary extract activates the endocrine glands related to the anterior lobe, namely, gonads, thyroid, and probably adrenals.
2. The increased secretion of these activated glands in turn stimulates the anterior lobe which augments with its own secretion



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## DESCRIPTION OF PLATES

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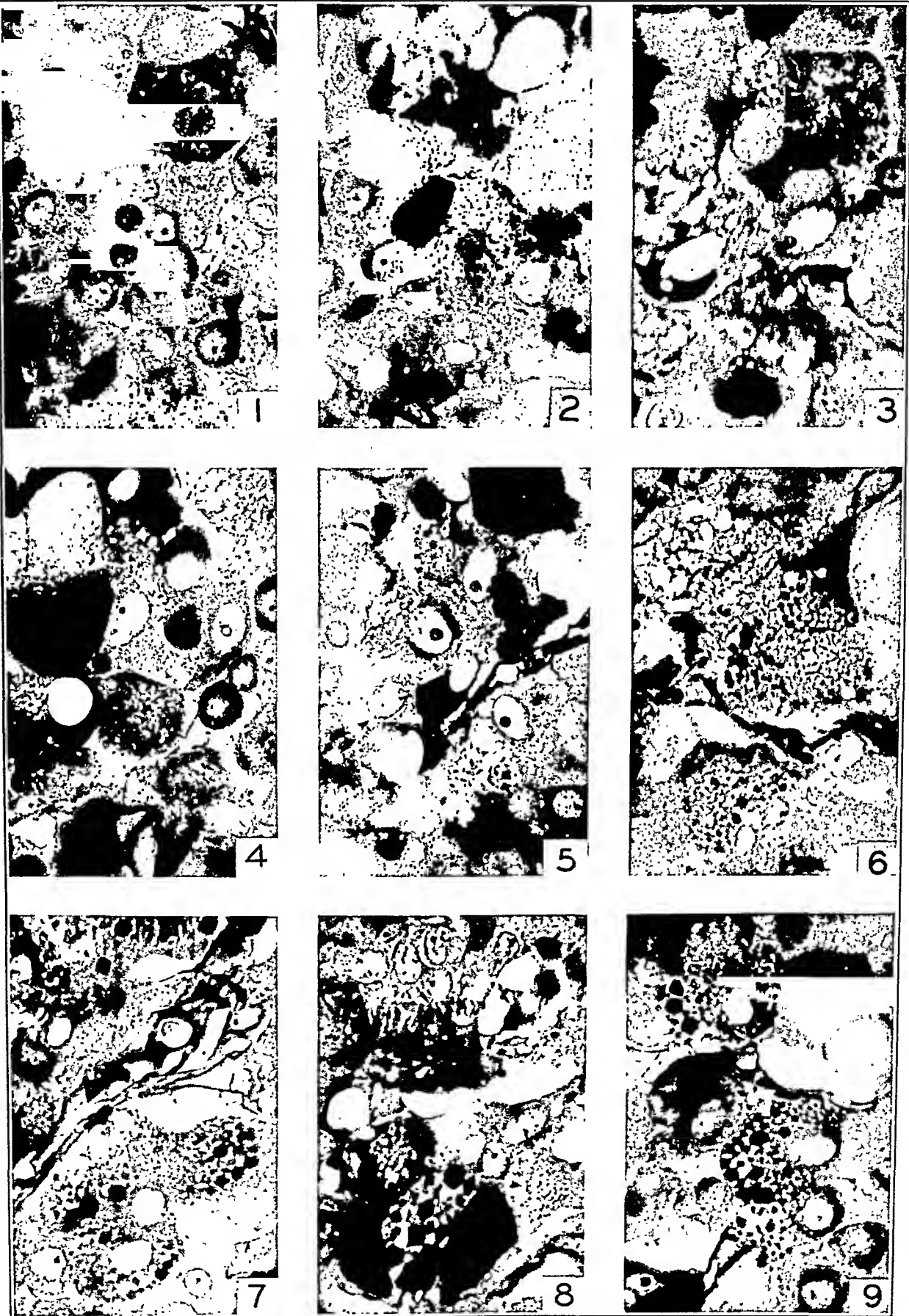
### PLATE 67

None of these photographs is retouched. They were taken at a magnification of about 950 X, unless otherwise designated.

FIGS. 1-4 show the clumping of basophilic granules into irregular masses. The dark masses are large colloid vacuoles. The clear vacuoles are also seen in various stages in Figs. 2 and 4.

FIGS. 5-9 inclusive show various stages of the transitional formation of dark granular masses into colloid vacuoles. Note their general distribution throughout the cytoplasm. The cell borders frequently become indistinct.





## PLATE 68

FIGS. 10-12. These photographs show the presence of larger dark basophilic vacuoles and their formation from smaller ones. See especially cell in Fig. 12. Cells in the lower left of Fig. 10, and upper and center right of Fig. 11 show vacuoles of the paler basophilic substance.

FIGS. 13-16 inclusive show the association of all 3 types of vacuoles within a single cell. In Fig. 15 one large disintegrating basophil (chromophobe indenting on right) occupies almost the entire area below the capillary and to the right of the connective tissue fibers.

FIG. 17. A typical castration type of vacuolation is seen in the cell at the upper center.

FIG. 18. Large basophils whose borders are indistinct border on the sinusoids. Such basophilic masses of cells are common.

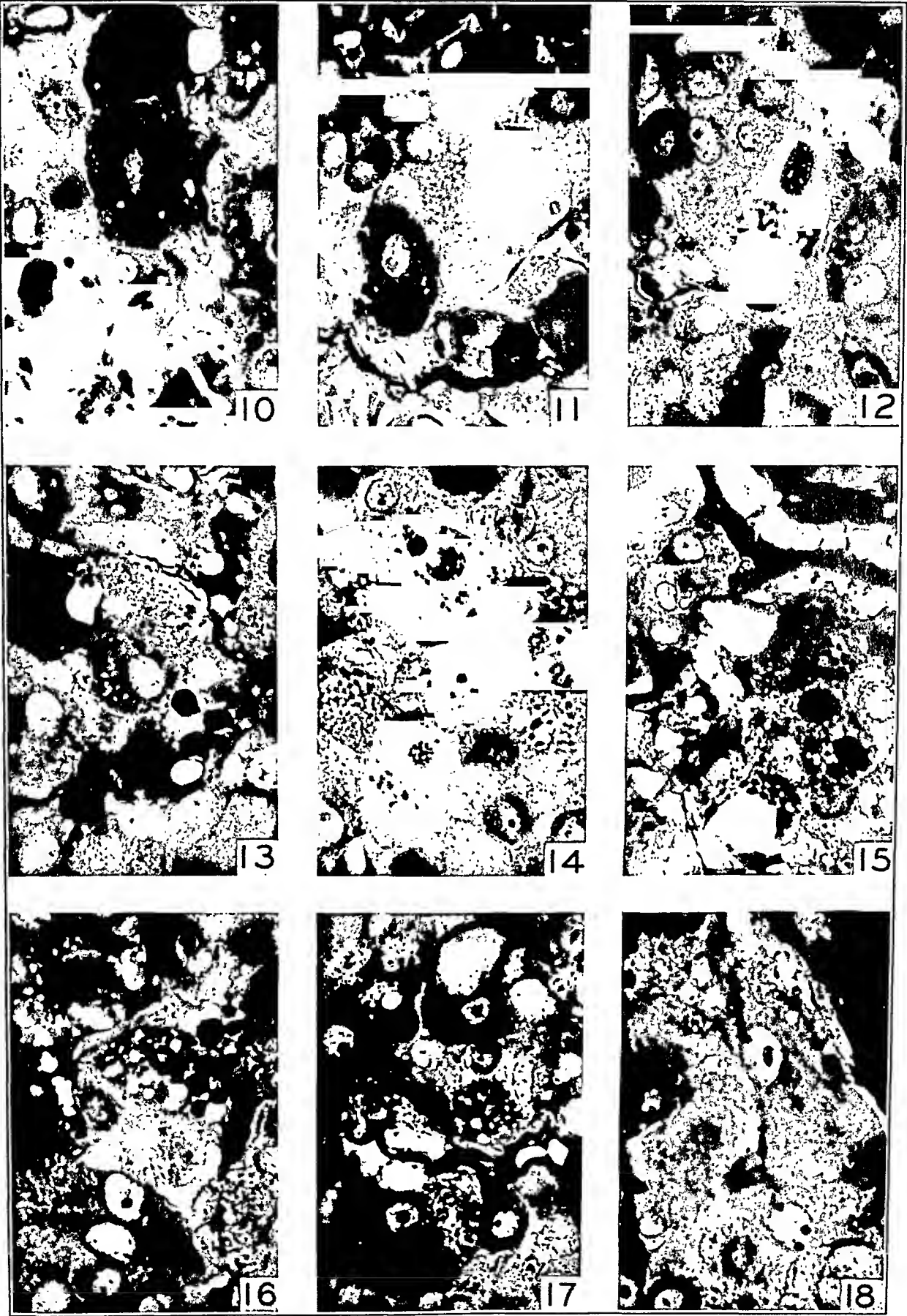


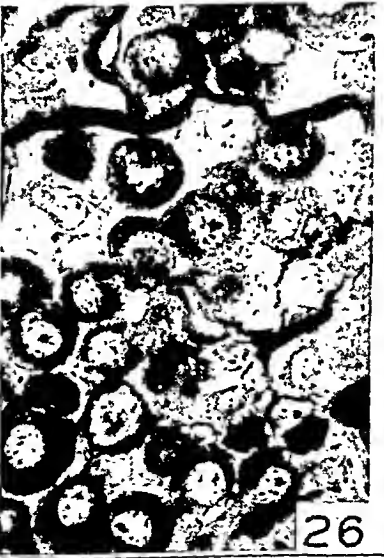
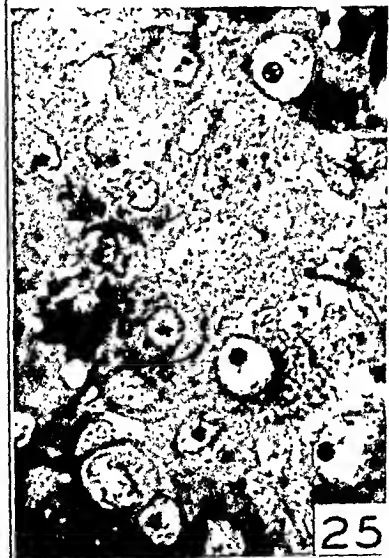
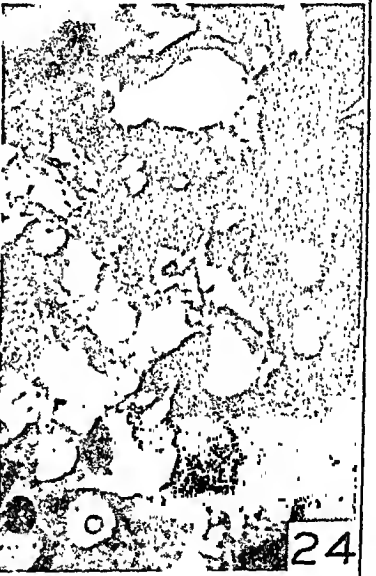
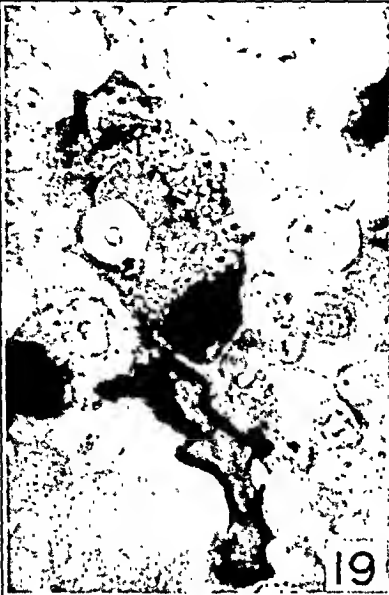
PLATE 69

FIGS. 19-24 show many stages in the progressive development of the clear vacuoles described in the text. With these are associated vacuoles of the other 2 types. These cells are typical of thyroidectomy changes in the basophils of the dog.

FIG. 25 shows a typical group of smaller basophils with fine cytoplasmic granulation and abundant mitochondria. These appear cytologically to be very active cells.

FIG. 26 shows a characteristic region of normal appearing acidophils.

FIG. 27 shows a characteristic region of pyknotic nucleated acidophils, an indication of cyclical secretory activity in the acidophils. See text.



## PLATE 70

FIG. 28 shows a typical area from the anterior hypophysis of a 10 months old lamb injected daily for 6 months with sheep pituitary extract. The dark granulated cells are acidophils. Homogeneous black areas in the cells are hyalinized portions of the cells. The light areas are largely degranulated basophils with a sparse grayish cytoplasmic granulation. About  $\times 550$ .

FIG. 29 shows a control lamb hypophysis. Note the distinct basophilic (deep blue) granulation of the basophils. Only color photography could indicate the marked contrast of these two glands. About  $\times 550$ .

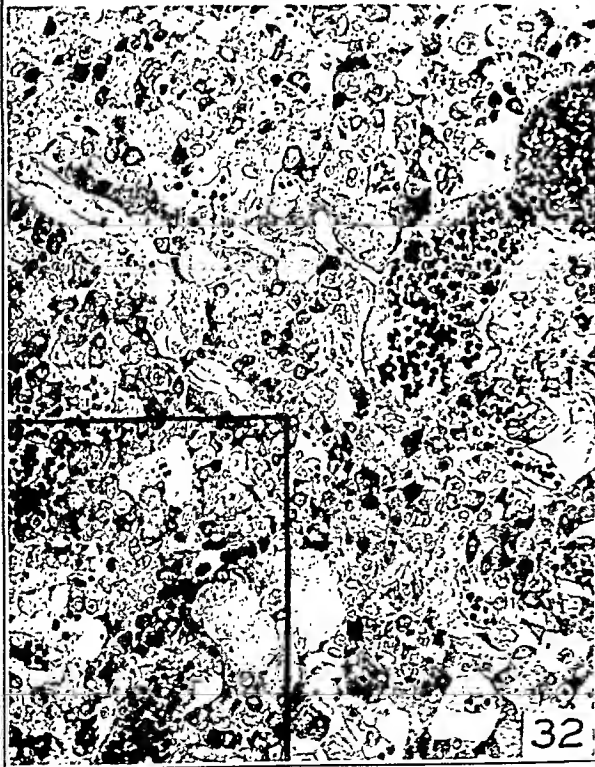
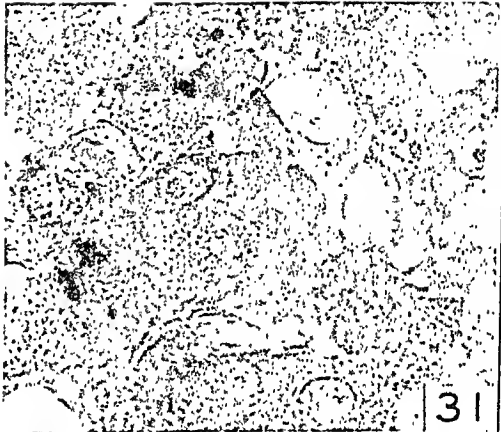
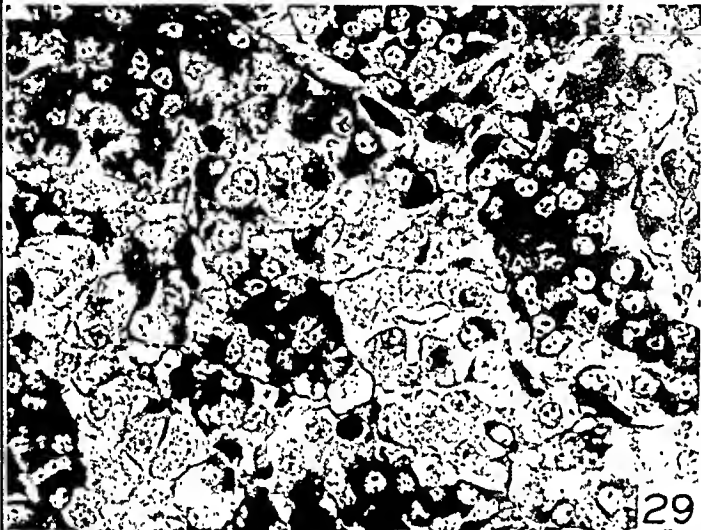
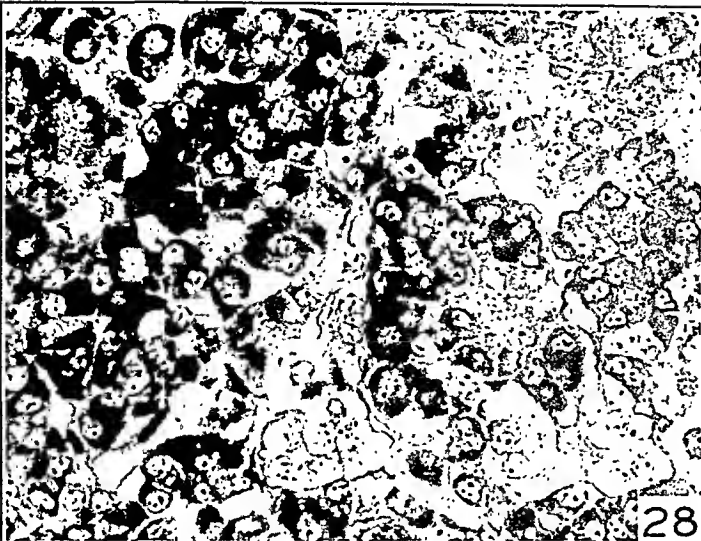
FIG. 30 shows actual degranulation of the bordering basophils into a distended sinusoid. Note the granular substance continuous across the sinusoid wall just to the right of the large central basophil. About  $\times 2000$ .

FIG. 31. The large basophil in the center has cytoplasmic granulation adjacent to and below the nucleus, but the periphery of the cell has been completely hyalinized. The striking similarity to Crooke changes is obvious.  $\times 2000$ .

FIG. 32. Typical area of a pituitary from an antihormone-injected animal. Note the extreme hyperemia and edema. A distended sinusoid is seen at the upper right, while at the lower right and left are typical contracted capillaries within edematous areas. Note the extravasation of red cells above the capillary in the edematous area and at the left center of the figure.

FIG. 33. High power field of square in Fig. 32. In the upper left the capillary, with its distinct endothelial nuclei, lies in an edematous space which is filled with red blood cells. Numerous examples such as this give evidence of widespread communication of the capillaries with the spaces. Note that the distended capillaries approximate in a cross section area the edematous spaces.









# THE LYMPHOCYTE IN ACUTE INFLAMMATION \*

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## INTRODUCTION AND REVIEW OF THE LITERATURE

The attitude of many histologists and pathologists toward the function of the lymphocyte is excellently summarized by Rich<sup>1</sup> in his general review of inflammation in resistance to infection. He states: "I am sure that all who are engaged in the study and teaching of pathology will agree that the complete ignorance of the function of this cell is one of the most humiliating and disgraceful gaps in all medical knowledge. . . . Literally, nothing of importance is known regarding the potentialities of these cells other than that they move and that they reproduce themselves."

The explanation of the rôle of the lymphocyte in tissue reactions accepted currently by teaching pathologists may be illustrated by a quotation from the latest edition of Boyd's Textbook of Pathology<sup>2</sup>: "In chronic inflammation and in the later stages of acute inflammation the lymphocyte may be the main cell of the exudate. The cells of such collections are often called by the non-committal name of 'small round cells.' This term is conveniently non-committal as to the origin of the small cells. It appears probable that the majority of the small round cells are derived from the tissues rather than from the blood. The lymphocyte of the blood has very little cytoplasm, and is therefore only slightly amœboid and not at all phagocytic for bacteria. It migrates from the vessels with difficulty, and much later than the polymorphonuclears. It is rather remarkable that we are so ignorant as to the exact rôle played by a cell which plays so dominant a part in the chronic infections."

MacCallum<sup>3</sup> in his textbook of pathology writes: "The mononuclear cells, which cannot be recognized as lymphocytes, are larger and assume a great variety of forms and sizes, so that they may come to be veritable giant-cells, often with many nuclei, and still one can draw no sharp line anywhere to divide them into

\* Received for publication March 22, 1939.

groups. Maximow, indeed, was sure that they all grew out of the lymphocytes which had emigrated from the blood-vessels. They are normally present in a great many places, not assembled in definite nodules of coherent tissue, as in the lymph-nodules, but scattered loosely in the mucosa of the whole intestinal tract, in the adventitial tissue of blood-vessels, in the loose tissue about the bronchi or the ureters — indeed, in the loose tissue anywhere in the body. At any rate they arrive very promptly in great numbers in any such tissue, if an occasion arises in which they are required to carry on for a time a phagocytic activity — to clean up the débris of cells. They are not easy to describe. From their motions when alive they seem very different from lymphocytes and it is hard to believe that those sluggish cells could have grown into these which reach out so eagerly and swallow fragments of cells so greedily. When they are fixed and stained they lose these characteristics and appear as rather large cells growing somewhat paler as they increase in size with vesicular nucleus with scattered chromatin particles. They are evidently the same cells as the monocytes of the circulating blood and they are the cells which have been variously called clasmotocytes, adventitial cells, macrophages, histiocytes, reticulo-endothelial cells, endothelial leucocytes, polyblasts, etc. We have hitherto called them mononuclear wandering cells and, since this is quite non-committal, we may well go on with it.”

Muir <sup>4</sup> states: “It can hardly be said that they (lymphocytes) have no phagocytic power, but this is at a minimum. Probably they become enlarged when they are going to exert their function. . . . They probably represent a response to the mildest type of irritation, but their precise function is not known. They migrate but are more concerned with chronic inflammation.”

We suggest that the uncertainty of the pathologists finds its basis in the usually confusing and often indefinite statements found in the histology books on the potentialities and functions of the lymphocyte. Cowdry <sup>5</sup> writes: “Emigration of lymphocytes takes place in a wide variety of conditions which have the single feature in common that they are of longer standing (chronic) and less temporary (acute) than those which lead to the mobilization outside of blood vessels of neutrophiles.” Bailey <sup>6</sup> states: “The lymphocytes migrate through the walls of the capillaries and dis-

play marked motility in the connective tissue. Their function is not clear but apparently they can develop into plasma cells and monocytes." Bremer<sup>7</sup> concludes that "lymphocytes are only infrequently phagocytic to vital dyes."

A second reason for the uncertainty of pathologists regarding this question is their lack of correlation of morphological modification of the lymphocyte with its functional variability. Aschoff<sup>8</sup> in discussing the ability of the lymphocyte to hypertrophy and become a phagocyte said, "I can only say there is no certain proof for such a transformation."

Metchnikoff,<sup>9</sup> probably because he was confronted with less complex situations than Aschoff, assumes a more familiar attitude toward these cells which is reflected in his statement: "The smaller white corpuscles found in fairly large numbers in the blood and the lymph and which are commonly known as lymphocytes or small lymphocytes are simply leucocytes with very little protoplasm which in this state never fulfill phagocytic functions. It is only when it becomes older, when its nucleus, single and rich in chromatin, becomes surrounded by an ample layer of protoplasm, that the lymphocyte becomes capable of ingesting and resorbing foreign bodies. Several authors, with Ehrlich at their head, still assign to these larger cells the same name — lymphocytes. Others however, give them the name of large mononuclear cells. Confusion is thus possible, especially as Ehrlich includes under the same term the large mononucleated leucocyte, a very rare form of cell in human blood which is distinguished by the greater staining capacity of its nucleus. To avoid this inconvenience I propose to designate the large lymphocytes by the name of blood macrophages and lymph macrophages (haemomacrophages, lymphomacrophages). . . . The mesoblastic phagocytes of the vertebrata are divided then, into fixed phagocytes — the macrophages of the spleen, endothelia, connective tissue, neuroglia, and muscle fibers — and free phagocytes. These latter are sometimes haemo- or lymphomacrophages, sometimes microphages. The fixed macrophages and the free macrophages resemble one another so greatly that it is very often extremely difficult, if not impossible, to differentiate them. For this reason it is often very useful, when the exact origin of a large phagocyte is not known, simply to name it macrophage."

Since Metchnikoff's description of the transformation of the lymphocyte to a macrophage much experimental morphological confirmatory evidence has accumulated.

Maximow<sup>10-17</sup> studied the problem of the function of the lymphocyte very extensively. His views are expressed in the following quotation from his Textbook of Histology. "The question of the prospective potencies of the small lymphocytes is of special interest. Many investigators believe these cells to be specifically differentiated elements incapable of further development. It has been conclusively shown, however, that some of the polyblasts in all inflammatory lesions arise from the local fixed macrophages, but that a much more abundant and important source is the lymphocytes and monocytes of the blood. These agranulocytes migrate from the blood vessels into the tissue, undergo here a rapid hypertrophy and are transformed into large phagocytic elements. In the first two days after the onset of inflammation they can still be distinguished from the polyblasts of local fixed macrophage origin by their smaller size. But, as they continue to increase in size, after two days or more the polyblasts of local and of hematogenous origin can no longer be distinguished. The differentiation of the polyblasts from the two sources becomes all the more difficult as the hypertrophied lymphocytes and monocytes very soon begin to store vital dyes if the inflammation occurs in a vitally stained animal."

Among the investigators who have confirmed the observations of Metchnikoff and Maximow, we wish to note the following: Ziegler<sup>18</sup> who concluded in his studies of edema of the skin and subcutaneous tissues that lymphocytes wander out of the blood and lymph vessels into an area of inflammation where they undergo morphological changes and become phagocytic; Schwarz<sup>19</sup> who noted that 2 to 10 hours after the onset of an acute inflammatory process the lymphocytes made up a part of the cellular exudate and later transformed into large mononuclears; Helly<sup>20</sup> who, after studying the morphology of exudate cells in acute inflammations produced experimentally by the anthrax bacillus, staphylococcus, streptococcus, the typhoid and the colon bacillus, cholera vibron, diphtheria bacillus, pneumococcus and the tubercle bacillus, concluded that the heterophils formed the microphages and the lymphocytes formed the macrophages in the inflammatory exu-

dates; Fischer <sup>21</sup> who found that about 7 hours after the introduction of irritants into the connective tissues of rats and mice, the lymphocytes that migrated into the area were changing into polyblasts; Tschaschin <sup>22, 23</sup> who supported Maximow's observations to the letter; Downey <sup>24</sup> who demonstrated that lymphocytes would take up colloidal dyes if the dyes were available; Bergel <sup>25</sup> who showed lymphocytes to be phagocytic for fats injected into the peritoneal and pleural cavities of guinea pigs and rabbits; Danchakoff and Seidlin <sup>26</sup> who observed that in the mesenchymal plate of the tail of a tadpole into which edestin was injected, lymphocytes migrated from the blood vessels and hypertrophied and gradually transformed into typical histotopic wandering cells or lymphoid phagocytes; and Stilwell <sup>27</sup> who observed inflammatory processes in the living frog's tongue. She injected diluted India ink intravenously and then injected egg yolk into the tongue and watched the local inflammatory process. The lymphocytes migrated at about 7 hours, hypertrophied and became phagocytic. Lang <sup>28</sup> concluded from a series of experiments on acute inflammation that lymphocytes and monocytes hypertrophy and become polyblasts. Bloom <sup>29, 30</sup> observed lymphocytes taken from the thoracic duct lymph transform into polyblasts between 7 and 20 hours after they were cultivated in tissue culture. Michels and Globus, <sup>31, 32</sup> in a study of the "so-called round cell infiltrations," found transitions between lymphocytes and macrophages. Watson <sup>33</sup> observed transitions from lymphocytes to phagocytic cells in the tissues of a patient dying of histoplasmosis. Ekola <sup>34, 35</sup> also demonstrated that lymphocytes transformed into macrophages. She studied connective tissue reactions resulting from the subcutaneous injection of various irritants such as sodium ricinoleate, trypan blue, diphtheria toxin and diphtheria soap vaccine. As early as 9 hours after the onset of inflammation she found lymphocytes changing into polyblasts. In the 1 and 2 day stages in her series polyblasts were easy to find. Higgins and Palmer <sup>36</sup> concluded that the lymphocytes could differentiate into histiocytic elements (macrophages) in experimentally produced hematomas. Stieve <sup>37</sup> observed that lymphocytes furnish part of the macrophages in the walls of inflamed human uteri, and Silberberg <sup>38</sup> found lymphocytes forming polyblasts (macrophages) in experimentally aseptically inflamed connective tissue.

From a survey of the above literature it is evident that an important part in the formation of the acute inflammatory cellular exudate has been ascribed to the lymphocyte. However, this function of the lymphocyte is not generally accepted by the authors of the current textbooks of pathology. We suggest this discrepancy is the result of the observation by pathologists of only the later stages of the acute inflammatory process at which time cell lineage is no longer discernible. Bloom<sup>39</sup> pointed out that since acute inflammation is such a dynamic phenomenon, it must be studied with dynamic technics. Above all, the first few hours after the onset of an inflammatory reaction must be studied carefully in order to obtain a true picture of the genesis of the process.

### MATERIALS AND METHODS

We present a series of experiments which, while adhering to Bloom's tenets, employ an original technic that allows the simultaneous demonstration of histogenous and hematogenous cellular elements contiguous in respect to time and environmental variation during the process of inflammation.

Young adult rabbits were used in our experiments. The hair was removed from the sides of the animals by shearing followed by shaving. A chemical depilatory was purposely avoided because of the possibility of cutaneous irritation.

An initial control biopsy is made prior to the injection of the irritant.

The irritant is injected subdermally at points 2.5 cm. to 4 cm. apart in the bare area. Egg albumin was found to be the most satisfactory irritant for our experiment. The optimal amount to inject was found to be from 40 to 80 ml. (mm.<sup>3</sup>). Greater amounts than this produce excessive edema which interferes with subsequent preparation of removed tissues. Amounts less than this do not produce a maximal cellular response. The point of injection is marked by a bland skin stain, such as methyl violet, because the inflamed areas are difficult to find. A map of the injected area is made and the position and time of the injection is recorded.

Adequate sampling of the inflamed tissue for biopsy can be obtained by removal of areas 1, 2 and 4 hours after injection. Tissue is then removed at 4 hour intervals through the 1st day,

and subsequently daily biopsies are obtained until healing is complete.

Under aseptic conditions the skin and subcutaneous tissue over the inflamed area is incised. A small amount of the loose connective tissue is picked up with forceps and excised. This tissue is transferred to a slide, spread out into a thin layer with teasing needles and dried quickly by whipping through the air. Several spreads may be made. The skin and subcutaneous tissue are then sutured.

These tissue spreads from now on are treated like blood smears. A May-Grünwald-Giemsa staining combination is employed: 30 drops of the May-Grünwald stain are put on the spread for 1 minute. This stain is then diluted with an equal number of drops of distilled water buffered to pH 6.4 and allowed to remain 4 minutes. The slide is then drained and flooded with the Giemsa stain 1.5 strength (1.5 drops of Giemsa stain per 1 cc. of the buffered water). This remains for 8 minutes when the preparation is differentiated in distilled water and blotted dry.

The advantages of these dry spreads of the loose connective tissue over wet spreads or sections are the same as those pointed out by Kirschbaum and Downey<sup>40</sup> for hematopoietic tissues, namely, (1) a marked improvement in cytological detail, and (2) a basis of comparison between these and the cells seen in dry smears of blood. The disadvantage of this method is that in the tissue spreads areas may be too thick and some selection of fields is required. Also, cell structure is retained at the expense of tissue architecture.

Our technic may be summarized as follows: Successive biopsies of inflamed tissue are fixed by drying in the air and are then stained by the methods commonly employed in hematological studies — staining methods that give the best cytological detail and allow a comparison of recognizable hematogenous elements with histogenous elements.

## RESULTS \*

We wish to limit ourselves as far as possible in this report to the behavior of the lymphocyte in the inflammatory process. We

\* The morphological studies discussed were made under the supervision of Dr. Hal Downey.

recognize that the initial response of a tissue varies somewhat with the character of the foreign body producing the inflammation. Egg albumin with colloidal carbon or starch precipitates a deluge of pseudoeosinophils (polymorphonuclears) in the rabbit. However, egg albumin alone produces an essentially characteristic and less complex reaction and is, therefore, more satisfactory for following the lymphocytic response to inflammation.

In a morphological study of the cellular constituents of an inflamed area in connective tissue the histogenous and hematogenous components must be considered separately. The histogenous cells are largely clasmatoocytes and fibroblasts which appear in approximately equal numbers together with occasional wandering lymphoid elements. Ranvier<sup>41</sup> introduced the name clasmatoocytes for the potential phagocytes of the connective tissue. These cells were called fixed macrophages by Metchnikoff,<sup>10</sup> rhagiocrine cells by Renaut,<sup>42</sup> adventitial cells by Marchand,<sup>43</sup> pyrrhol cells by Goldmann,<sup>44</sup> resting wandering cells by Maximow<sup>11-14, 17</sup> and histiocytes by Aschoff and Kiyono.<sup>45</sup>

The hematogenous cells seen are granular leukocytes including the pseudoeosinophils (polymorphonuclears), the eosinophils and the basophils, as well as the non-granular leukocytes consisting of lymphocytes and monocytes.

The cells that receive our attention in this study are the clasmatoocytes and lymphocytes, both of which can assume a phagocytic function.

We present our results by describing a series of cells from tissue removed for biopsy taken in a time sequence from experimentally produced inflammatory areas in rabbits.

The initial control biopsy contains clasmatoocytes (Fig. 1). These are large round, oval or elongated cells. With our technic the nuclei of these cells show a rather coarse, sieve-like chromatin pattern. The chromatin granules are usually of a uniform size. However, occasionally condensation occurs resulting in the formation of clumps. The lavender parachromatin is sharply demarcated from the purple chromatin. The nuclear membrane is relatively thick and nucleoli occur infrequently. The nucleus is surrounded by a mildly basophilic, mottled, poorly outlined mass of cytoplasm. The granular basophilic spongioplasmic network contains many small, clear hyaloplasmic vacuoles. In some of these cells small



acidophilic and darkly basophilic cytoplasmic inclusions are seen. Occasionally in the cytoplasm large vacuoles are encountered.

The first 12 hours following the initiation of the acute inflammatory process the histogenous macrophage response overshadows the activity of the hematogenous macrophages. In this same period the activation of the clasmatocytes is accomplished. During the process of clasmatocytic activity there is little discernible change in nuclear architecture, so our description is largely concerned with cytoplasmic changes. Two hours after the injection of egg albumin the clasmatocytes (Fig. 2) show numerous large hyaloplasmic vacuoles containing various sized acidophilic inclusions. Although the extracellular albumin stains basophilic with the May-Grünwald-Giemsa combination, we assume the acidophilic cytoplasmic inclusions in the clasmatocytes are ingested egg albumin. From this stage on the clasmatocytes will be referred to as histogenous macrophages since this term indicates their source and function. We note that the number of clasmatocytes at this time has not increased.

At the 2 hour stage the hematogenous cellular response is indicated by the appearance of an occasional pseudoeosinophil, a cell homologous to the neutrophil of man. However, it is obvious that the first line of defence is by histogenous rather than hematogenous elements and the cell to react first is the clasmatocyte.

At the 4th hour of acute inflammation the tissue macrophages show the same changes as at the 2nd hour. The cytoplasm contains acidophilic inclusions of various sizes in great quantities. The hematogenous cells are slightly increased in number between the 2nd and 4th hours.

Eight hours after exposure to albumin the histogenous macrophages contain metachromatic granules in addition to the acidophilic cytoplasmic inclusions. Because of transition stages present, these granules are assumed to be a further step in the digestion of phagocytosed albumin. Migrating non-granular blood cells are beginning to appear after 8 hours of inflammation.

The 12th hour of the inflammatory process shows an increasingly frequent presence of metachromatic inclusions in the cytoplasm of the histogenous macrophages. Now for the first time the blood cells are beginning to approach the tissue cells in respect to numbers present. The pseudoeosinophil is a common cell and an

occasional eosinophil is seen. However, while the non-granular leukocytes, especially the lymphocytes, are not as conspicuous as the granular leukocytes, they are as numerous.

By the 14th hour (Fig. 3) the hematogenous elements outnumber the histogenous cells in the inflamed area, and the most frequently appearing hemic leukocyte is the lymphocyte. The histogenous macrophages still retain the typical clasmatocyte type of nucleus. Their cytoplasm still contains large numbers of acidophilic and metachromatic inclusions.

The lymphocytes (Fig. 8a) are small, medium and large. They have the characteristic pachychromatic nucleus in which the chromatin-parachromatin separation is not distinct. The narrow rim of cytoplasm in these cells is intensely basophilic due to a relatively small amount of yellow hyaloplasm as compared to the blue spongioplasm. Azurophilic inclusions are sometimes found in the hyaloplasm.

At this time — 14 hours after induction of the inflammatory process — indications of the lymphocyte transformation are well established. The pachychromatic lymphocytic nucleus becomes more diffuse. The chromatin becomes sharply demarcated from the parachromatin which shows a relative increase in amount. The chromatin blocks fragment and the nucleus assumes a more leptochromatic appearance. The end result of this transformation is the development of a small mononuclear cell whose nucleus bares little indication of its ancestry. This then — between the 8th and 14th hour — is the critical period in the evolution of the hematogenous exudate in acute inflammation. Any exposition of the inflammatory process which disregards the only opportunity to determine cell ancestry is open to serious criticism. At 8 hours the inflamed area shows many typical small lymphocytes — by the 14th hour all stages of transition between the trachychromatic lymphocyte nucleus to the more amblychromatic nucleus common to hematogenous macrophages are present.

The cytoplasm of the lymphocyte increases in amount and becomes less basophilic. This is brought about by an increase in the hyaloplasm with a resultant dispersal of the spongioplasm. Cells still containing azurophilic granulation may show a phagocytosed erythrocyte, acidophilic albuminous droplet or a degenerated pseudoeosinophil. Some of the metamorphosing lymphocytes show

small cytoplasmic pseudopodia. Since these cells have lost their pachychromatic nucleus and intensely basophilic cytoplasm — characteristics on which the recognition of lymphocytes depends — we shall refer to them as hematogenous macrophages (Fig. 8b).

At 18 hours (Fig. 4) the histogenous macrophages do not show any further changes. These cells are outnumbered by the hematogenous macrophages. The hematogenous macrophages are round or oval, moderately basophilic cells, much smaller than the histogenous macrophages. The nuclear and cytoplasmic hypertrophy is more marked than formerly and the phagocytic activity of these cells is more evident (Fig. 9).

The nucleus shows a somewhat coarser pattern in the hematogenous macrophage at this time than the nucleus of the histogenous macrophage.

By 26 hours (Fig. 5) the fibroblasts are showing mitosis. The histogenous macrophages have digested their acidophilic inclusions and consequently show only the metachromatic granules. Their vacuoles have decreased in size and the spongioplasm being more compact gives the cell a basophilic appearance.

The hematogenous macrophages now have a chromatin pattern similar to that of histogenous macrophages, but similarity to the lymphocyte nucleus is not apparent (Fig. 10). The cytoplasm of the hematogenous macrophages is still increasing and cell inclusions — vacuoles, albumin, pseudoeosinophils, and so on, are seen.

At the 49th hour (Fig. 6) the fibroblasts showing mitosis are the most conspicuous cells. However, they show no tendency to form definitive macrophages. The histogenous macrophages are becoming more basophilic and somewhat smaller. The presence of metachromatic granules indicates a previous phagocytic activity. The hematogenous macrophage nucleus does not show further changes. The cells are still smaller than the histogenous macrophages.

At 76 hours (Fig. 7) the histogenous and hematogenous macrophages approximate each other in size and morphology. They are both large mononuclear cells with comparatively leptochromatic nuclei and basophilic cytoplasm. Both cell lines show phagocytosis of erythrocytes, pseudoeosinophils, and so on (Fig. 11).

## DISCUSSION

Both acutely and chronically inflamed tissues possess a positive chemotactic attraction for hematogenous lymphocytes. In acute inflammatory exudates, in contrast to their retained lymphocytic morphology in chronic infiltrations, the lymphocytes become macrophages.

The transformation of a lymphocyte to a macrophage involves a marked alteration in nuclear structure as well as cytoplasmic and functional modifications. The factors underlying these changes are not clear. However, we wish to point out that the transformation of the pachychromatic lymphocytic nucleus to the larger, more leptochromatic nucleus of the macrophage, would not be incompatible with the development of a nuclear edema. The change in the nucleus is an increase in parachromatin and a decrease in size of the chromatin blocks. Even the increased chromatin-parachromatin definition could be produced by an increase in the nuclear sap, with a subsequent less dense chromatin. This edema we would associate with the disturbance of osmotic pressures due to a change in the pH of the intercellular fluids associated with acute inflammation. Certainly, in chronic lymphocytic infiltrations where these changes are less marked the lymphocyte nucleus retains its identity.

The cytoplasmic changes in the lymphocytes are the result of two not intimately associated processes. There is an increase in the hyaloplasm which may be the result of imbibition which by separating the particles of the blue spongioplasm gives us a cell with a moderate increase in cytoplasm with a decreased basophilia (Figs. 8, 9, 10). The cytoplasm of the hematogenous macrophage undergoes a quasihypertrophy by the phagocytosis of pseudo-eosinophils, erythrocytes or albumin (Figs. 11, 12). It is only when the cytoplasmic edema is accompanied by phagocytosis that these cells obtain their maximum size.

Our studies tend to confirm the observation of Hertzog<sup>46</sup> that the phagocytic ability of a cell varies with its cytoplasmic mass.

We have in part at least answered Opie,<sup>47</sup> who in 1910 wrote: "If it were possible to define the origin of the mononuclear cells concerned in the inflammatory reaction of all vertebrate animals as well as it is possible to define the character and sources of the

common polynuclear leucocytes concerned in the same phenomenon, it might be possible to describe with an accurate generalization the essential nature of the cellular accumulation which follows the action of substance foreign to a tissue."

### CONCLUSIONS

1. The transformation of clasmatoocytes to histogenous macrophages is the initial response of the rabbit in acute inflammation.
2. The majority of the macrophages in the exudate associated with the acute inflammatory process are of hematogenous origin.
3. The lymphocyte-macrophage transformation occurs early in the course of the inflammation. By the 14th hour the lymphocytic origin of many mononuclear cells in an inflamed area is largely obscured. In studies made 18 hours or later after the onset of an acute inflammation in a tissue, cell lineage cannot be traced.
4. The employment of tissue spreads, dried and stained like blood smears, allows a comparison of the cells in an acutely inflamed tissue with cells of blood smears.

NOTE: I am indebted to Mr. Henry W. Morris for the microphotographs.

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## DESCRIPTION OF PLATES

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### PLATE 71

- FIG. 1. Normal clasmatoctes from the subcutaneous connective tissue.  $\times 1100$ .
- FIG. 2. Biopsied connective tissue during acute inflammatory stage at 2 hours. Activated clasmatoctes showing phagocytosis (histogenous macrophages).  $\times 1200$ .
- FIG. 3. Acute inflammatory stage at 14 hours. a = Histogenous macrophage; b = lymphocytes.  $\times 1100$ .
- FIG. 4. Acute inflammatory stage at 18 hours. a = Histogenous macrophage; b = hematogenous macrophages (hypertrophied lymphocytes).  $\times 1100$ .





PLATE 72

FIG. 5. a = Histogenous macrophages with metachromatic granulation; b = hematogenous macrophages (hypertrophied lymphocytes). Nuclear similarity of macrophages is now evident. c = Fibroblast undergoing mitotic division at 26 hours.  $\times 1100$ .

FIG. 6. a = Histogenous macrophages; b = hematogenous macrophages at 49 hours.  $\times 1100$ .

FIG. 7. a = Histogenous macrophages; b = hematogenous macrophages; c = fibroblast. Note similarity of macrophages at 76 hours.  $\times 1100$ .

FIG. 8. Illustrating detail of lymphocyte-hematogenous macrophage transformation at 14 hours. a = Lymphocytes; b = early stage showing less compact nuclear structure.  $\times 1500$ .

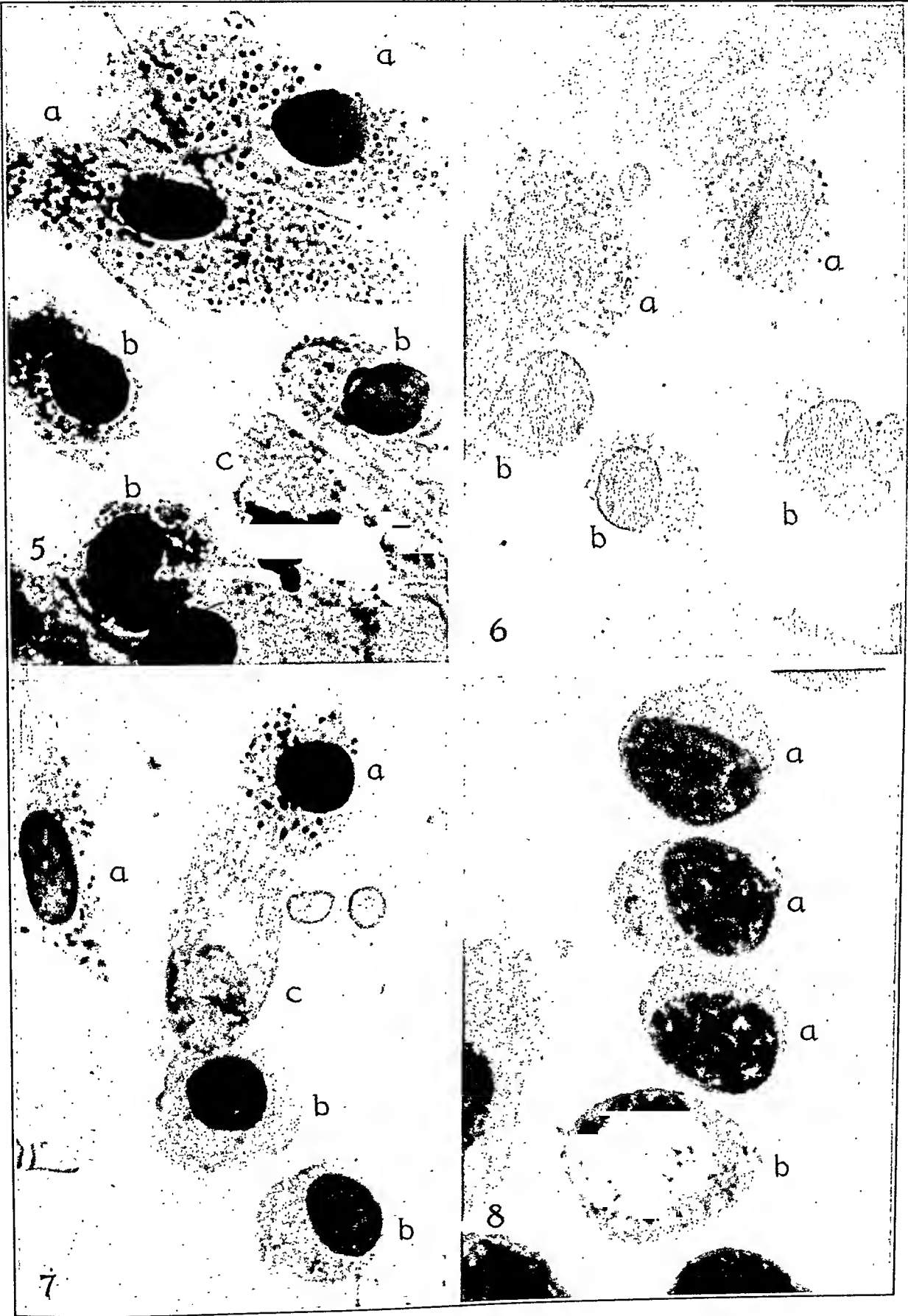


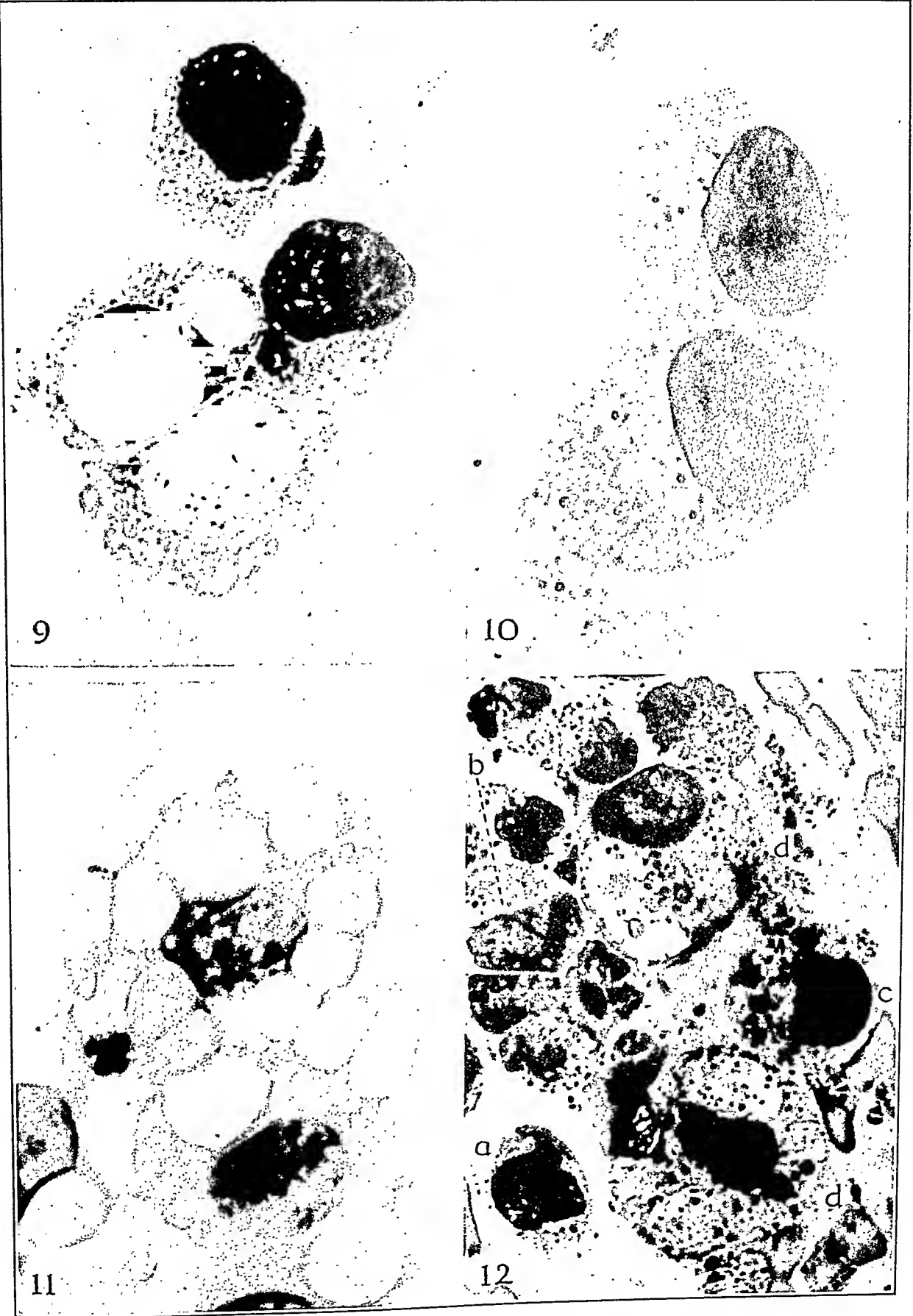
PLATE 73

FIG. 9. Hematogenous macrophages at 18 hours showing an increase in size, a decreased cytoplasmic basophilia and less compact nuclei than the lymphocytes from which they are derived.  $\times 1500$ .

FIG. 10. Hematogenous macrophages at 26 hours containing a few meta-chromatic granules. Maximal nuclear hypertrophy is now present but through phagocytosis the cytoplasmic volume may be further increased. At this time the lymphocytic ancestry of the cells is not apparent.  $\times 1500$ .

FIG. 11. Erythrocytic phagocytosis by hematogenous macrophages.  $\times 1500$ .

FIG. 12. a, b, c and d show correlation between cytoplasmic volume and phagocytic activity of hematogenous macrophages 38 hours following subcutaneous injection of starch.  $\times 1500$ .



The Lymphocyte in Acute Inflammation

Kolouch



PLASMA PROTEIN, BILE SALT AND CHOLESTEROL  
METABOLISM AS INFLUENCED BY MULTIPLE  
INJECTIONS OF GUM ACACIA IN BILE  
FISTULA DOGS \*

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INTRODUCTION

The liver <sup>1</sup> is the site of production of the bile salts and is the organ normally responsible for the elimination of bile pigments. It has been shown <sup>2</sup> repeatedly that such small amounts of chloroform as 3 cc. given by stomach tube on 3 or 4 successive days will cause a marked decrease in the amount of bile salt excreted by a bile fistula dog. The injury to the liver is slight and there is prompt repair with normal production of bile salts. In some of the dogs after slight chloroform injury there were only traces of bile salt and yet the volume of bile and the amount of bile pigments were little, if any, changed. Whether or not there will be a decrease in volume and bile pigments depends on the severity of the chloroform injury. Chloroform given as an anesthetic for 15 to 30 minutes causes a similar depression in the bile salts output. Injury to the liver effected by phosphorus (injected subcutaneously in olive oil) causes a similar reaction. When <sup>3</sup> the bile fistula is combined with the Eck fistula there results a decreased production in bile salt but the volume and amount of bile pigment in the bile are not affected. This lowered output of bile salt is undoubtedly related to the fact that the liver of the Eck fistula dog is atrophic and its function abnormal.

When <sup>4,5</sup> there is disturbance in liver function due to obstruction of the biliary tree or to parenchymatous injury, there is an alteration in the cholesterol of the blood. In the case of obstruction the total blood cholesterol becomes elevated but the esters of cholesterol do not increase in a parallel manner. With chronic chloroform injury produced by feeding the drug over an extended period, the total blood cholesterol decreases and the esters of

\* Received for publication March 10, 1939.

We are indebted to Eli Lilly and Company for valuable materials used in these experiments.

cholesterol may diminish actually to the vanishing point. Infection within the liver causes a similar reduction. It is apparent that we have some knowledge as to what may occur when there is disturbed physiology of the liver.

Andersch and Gibson<sup>6</sup> demonstrated that acacia when injected intravenously into dogs is removed and retained in large part in the liver cells. They reported that the bile salt production and bile pigment elimination were reduced. Heckel and co-workers<sup>7</sup> have recently shown that following repeated injections of acacia into the blood stream there results a marked decrease in the plasma proteins with profound reduction in the *plasma fibrinogen* levels. The decrease in fibrinogen from the normal of 300–350 mg. per cent to 50–75 mg. per cent is suggestive evidence that the acacia within the liver cells may be impairing their function at least as regards the production of fibrinogen.

In view of this fact, it was thought that possibly other activities of the liver might be deranged, particularly the bile salt and cholesterol metabolism.

The 2 bile fistula dogs used in these experiments received 4 and 6 doses of acacia by venous injection in 30 gm. amounts. Autopsy revealed that the livers were enlarged, had a glassy appearance with conspicuous lobules, and histologically the liver cells had the peculiar pale reticulated and vacuolated appearance associated with acacia deposition. The bile salt production, bile pigment and bile cholesterol elimination, and the blood cholesterol and plasma proteins have been followed. The plasma proteins decreased and on the days that followed the injection of the acacia there was noted constantly a temporary decrease in the bile salts, but otherwise there has been no effect produced as the result of the liver cells being filled with acacia. The dogs<sup>8</sup> handled the bile salts, when fed, in the normal manner, that is, the salts were absorbed and promptly excreted in the bile. Chloroform and carbon tetrachloride, when fed, caused the expected decrease in bile salt formation.

These data indicate that the acacia within the liver cells causes no serious impairment of their ability to form bile salts. Other experiments<sup>7</sup> point to the possibility that the acacia may interfere with the production of fibrinogen by the liver cells. Such dissociation of functions of the liver is not uncommon.



## METHODS

The closed sterile bag type fistula as devised by Rous and McMaster<sup>9</sup> and modified by Smith, Groth and Whipple<sup>10</sup> was used. The dogs were fed the salmon bread diet since it is one that is low in fat and rich in carbohydrates and therefore suitable for a dog totally deprived of bile. Its preparation has been described in detail.<sup>11</sup>

Bile salt determinations were made following the method<sup>12</sup> of Foster and Hooper and entail the estimation of the amino nitrogen as determined by the method of Van Slyke.

Methods for bile pigment,<sup>13</sup> bile cholesterol,<sup>14</sup> total blood cholesterol, and esters<sup>15</sup> determinations may be found in detail in previous papers.

Plasma proteins were determined by the macro-Kjeldahl method, using selenous acid as the oxidizing agent.

Hemoglobin was determined following the method described by Robscheit.<sup>16</sup> Standard is equivalent to 13.8 gm. of hemoglobin per 100 cc.

The gum acacia was furnished by the Eli Lilly Company in ampoules containing 30 gm. of acacia in 100 cc. of solution. The contents of an ampoule were mixed in 150 cc. of Locke's solution and injected intravenously from a gravity bottle.

## EXPERIMENTAL

Dog 36-9 was a mongrel female and was studied for a period of 123 days after the bile fistula was established. No bile was fed except during the periods indicated in the tables. After an adequate base line period, 30 gm. of acacia in Locke's solution were injected intravenously and determinations made for the following 27 days and then the same sized dose was repeated at intervals of 18, 6, 9 and 22 days. The dog after each injection of acacia would immediately vomit a small amount of mucoid material but within 5 minutes would be quite normal with no further disturbance. Periods of interest are given in the tables.

Three uncomplicated periods are illustrated in Table I. On the day after the 2nd injection of acacia there is a decrease in the bile salt production. The bile cholesterol is slightly lower on this day also and this may be related to the lessened production of bile salt. Wright and Whipple<sup>17</sup> have shown that the cholesterol in the bile

TABLE I  
Repeated Intravenous Injections of Acacia

Dog 36-9		Bile volume	Bile pigments	Bile salts	Bile cholesterol	Total blood cholesterol	Blood cholesterol esters	Plasma proteins	Hemo-globin	Weight
Date	cc.	mg.	mg.	mg.	mg.	mg./%	mg./%	gm./%	%	kg.
Nov. 24	172	83	2183	13.2	71	48	67	6.01	117	16.1
Second Injection of Acacia (30 gm.) on November 24th										
25	124	82	1449	9.8	73	48	66	4.92	...	...
26	164	99	2275	14.1	72	49	68	5.01	...	...
27	132	100	2000	14.8	59	42	71	4.99	...	16.1
28	156	87	1541	...	...	...	...	...	...	...
29	140	86	2073	17.3	56	39	70	5.12	117	16.2
Third Injection of Acacia (30 gm.) on December 12th										
Dec. 12	197	92	2036	...	...	...	...	5.54	...	...
13	180	106	1651	14.2	70	48	69	4.94	...	16.1
14	162	152	1780	13.2	52	41	79	4.48	102	...
15	182	151	2051	11.3	51	32	63	4.73	...	16.1
16	154	123	1780	14.7	56	35	63	4.66	...	...
17	190	125	2220	...	49	36	74	4.93	...	16.1
Fifth Injection of Acacia (30 gm.) on January 18th										
Jan. 18	206	79	2110	25.4	47	30	64	5.38	109	16.1
19	180	98	1651	11.2	52	32	62	4.25	...	...
20	180	121	1853	22.2	42	...	...	4.26	...	...
21	208	116	2550	19.6	42	29	69	4.62	...	...
22	232	98	2624	26.9	51	32	63	4.76	...	16.2

30 gm. acacia injected on October 28th, November 24th, December 12th, 18th and 27th, and January 18th. Total 180 gm.

fluctuates with variation in the bile salt content. Bile pigments and blood cholesterol levels are not altered but there is definite decrease in the percentage of circulating plasma proteins with return to normal levels by the 5th day after the injection. Normal values were obtained for the days following this period and so on December 12th a 3rd injection was given. The bile salt output on the following 2 days was again decreased but returned to normal levels. Blood and bile cholesterol values were not altered. The blood cholesterol, both total and esters, is now lower than in the fore period but the ester percentage remains the same. This lowering of blood cholesterol with maintenance of relation of total to esters of cholesterol is a phenomenon that always occurs in fistula dogs after a period of total bile deprivation. The plasma protein percentage dropped and on the 8th day was 4.25 and remained at this lower level and only after 21 days was there a return to a low normal level of 5.07 gm. per cent. The bile pigments were elevated after the injection. This is not a constant finding and possibly is related to a slight amount of red cell destruction. After the 5th injection of acacia there is again slight excess of bile pigment, some decrease in bile salt with immediate recovery, and an even higher level than on previous days. Bile cholesterol decreases on the day of low bile salt output, but the blood cholesterol shows no change of significance. The blood proteins decrease. It is important to note that the dog's weight has remained constant. The hemoglobin percentage is slightly lowered, but 15 cc. samples of blood were being removed daily. From these data it is apparent that the presence of the acacia in the liver has not interfered with the orderly elimination of bile pigment and bile cholesterol, or the production of bile salts. The fact that the blood cholesterol levels are normal indicates also that the acacia is causing no profound injury to the liver cells.

The effects of obstruction, chloroform feeding and bile salt feeding are clearly brought out in Table II. After the 4th injection of acacia the fistula tract became totally obstructed. On 3 days there were 50, 30 and 30 cc. of pale, watery green fluid and no determinations were performed on this so-called bile. The total blood cholesterol shows the effect of the obstruction as the total cholesterol increases markedly and the ratio of total to esters is slightly lowered. This is the expected reaction to obstruction. On

TABLE II  
Effects of Obstruction, Bile Salt and Chloroform Feeding on the Acacia Filled Liver

Dog 36-9

Date	Bile volume	Bile pigments	Bile salts	Bile cholesterol	Total blood cholesterol	Blood cholesterol esters	Blood cholesterol esters	Plasma proteins	Hemo-globin	Weight
	cc.	mg.	mg.	mg.	mg./%	mg./%	%	gm./%	%	kg.
Dec. 27	214	79	2330	6.9	45	34	76	...	..	16.2
Fourth Injection of Acacia (30 gm.) on December 27th										
28	150	97	1431	4.9	45	24	53	4.11	..	...
29*	50	40	550	...	60	36	60	4.29	..	16.2
30*	0	..	....	...	74	42	57	4.21	99	...
31*	30	16	....	...	119	67	56	4.73	..	16
Jan. 1	0	..	....	...	151	79	52	...	..	...
2	0	..	....	...	..	..	..	...	..	...
3*	30	..	....	...	..	..	..	...	..	...
4	320	148	1743	...	..	..	..	...	..	...
5	320	181	2752	17.3	38	22	58	4.83	..	...
6	290	150	2404	21.2	34	22	65	4.89	..	16.2
Bile by Mouth for 3 Days (January 14th, 15th and 16th)										
13	216	78	2257	26.0	43	27	63	5.46	..	16.1
14	260	75	3340	19.3	43	29	67	5.22	..	...
15	236	70	3486	29.2	43	27	63	5.26	..	16.1
16	255	79	3670	...	..	..	..	...	..	...
17	188	66	1982	22.9	50	37	74	5.63	109	16
Chloroform (3 cc.) by Mouth for 3 Days (January 26th, 27th and 28th)										
26	278	85	2624	...	51	32	63	5.09	..	16.1
27	252	89	2257	24.2	42	27	64	4.90	..	...
28	230	81	1046	11.4	49	31	63	5.00	..	...
29	204	79	991	12.0	58	34	59	4.80	..	16.1
30	282	101	1266	...	..	..	..	...	..	...
31	184	50	1101	11.0	66	35	53	5.12	..	...
Feb. 1	296	97	2642	20.4	58	39	67	5.35	..	...

\* Dog bile (75 cc.) by stomach tube.

the 4 days indicated in the table 75 cc. of whole bile were given by stomach tube in an attempt to cause an increased flow of bile. The rubber tubing was injected with sterile saline and suction applied also. Between the two procedures successful clearing of the fistula was accomplished as on the 7th day following obstruction a large volume of normal appearing bile was excreted. There was prompt recovery of the bile salt and bile cholesterol output and the blood cholesterol levels fell to the previous base line. Bile pigments were of course elevated. The value of bile feeding to cause an increase in bile volume and so aid in flushing out the fistula tract is well illustrated by these data. The obstruction may have been partially caused by acacia as it is known to be eliminated in the bile.

In the normal fistula dog bile salts, when fed, are absorbed and quantitatively excreted promptly in the bile. In order to see whether the liver cells packed with acacia would function in a similar manner, whole bile (100 cc.) was given by stomach tube on 3 successive days. These portions of bile contained 1006 mg., 1045 mg. and 1478 mg. of bile salt, respectively, or a total of 3529 mg. During a 10 day control period the daily output of bile salt averaged 2161 mg. and as the result of the bile feeding there was an increased elimination of 4013 mg. of bile salt so that there was complete excretion of the 3529 gm. fed. The bile cholesterol increased also, due to the increased amount of bile salts in the bile. Bile pigments and blood cholesterol are not affected. Again there is a normal reaction in spite of the acacia in the liver cells.

When chloroform (3 cc.) is given by mouth to the dog while its liver is still filled with acacia, we see that bile volume and pigments are not affected in the slightest but there is a successive decrease in the bile salt and bile cholesterol output with return to normal on the 4th day after the drug was stopped. Blood cholesterol is not affected as the injury to the liver by this small amount of chloroform is minimal and acute in character.

A week following the chloroform feeding experiment the fistula tract became obstructed and after 4 days the dog was killed with ether anesthesia followed by immediate autopsy. There was jaundice of the sclerae and mucous membranes. The serous cavities were normal except for some adhesions about the rubber tubing in the peritoneal cavity. The heart and lungs were normal

grossly and histologically. Blood clots were normal in appearance and consistence. The spleen was firm and red in color, with the malpighian bodies standing out distinctly as glistening gray nodules 1 mm. in diameter. Sections showed less numerous red cells and pulp cells but scattered throughout there were large phagocytic cells that were pale staining, frothy and vacuolated. These cells apparently had taken up some of the acacia.

The liver weighed 900 gm. and its capsule was smooth. Normally the liver of a dog of this size would weigh about 350 gm. Cut surfaces had a light yellowish brown cast due to staining with bile pigment. The lobules were large and regular and the gland had a glassy appearance due to the acacia. It was friable in consistence. The extrahepatic ducts were markedly dilated and the common bile duct measured 1.5 cm. in circumference. It contained whitish fluid with some flecks of green colored material. The cannula was in place within the duct but it was obstructed by dry, greenish inspissated bile.

Histological sections show inspissated pigment within the small bile canaliculi. The architecture is normal but the liver cells are large and they appear frothy, vacuolated or reticulated with no normal appearing cytoplasm. This abnormal appearance is due to the contained acacia. Sections of the liver when stained with sudan IV are found to contain no fat in the liver cells. The gastrointestinal tract, pancreas, adrenals, kidneys, ureters, bladder, uterus, tubes and ovaries are all normal. The bone marrow shows the normal distribution of marrow.

Dog 37-106 was a female mongrel Airedale and was followed for 84 days after the bile fistula was established. It received no bile except as indicated in the tables. After an adequate base line period, 30 gm. of acacia were injected intravenously with repeated injection at intervals of 8, 6 and 12 days. This dog also vomited immediately after each injection but recovered promptly with no further disturbance.

The two periods illustrated in Table III are typical of the reaction following each injection of acacia. After the 1st injection there is a slight decrease in the bile salts excreted on the day following the injection. The bile pigments and bile cholesterol are not affected nor is there any alteration in the blood cholesterol levels. However, there was prompt and marked decrease in the

TABLE III  
Repeated Intravenous Injections of Acacia

Date	Bile volume cc.	Bile pigments mg.	Bile salts mg.	Bile cholesterol mg.	Total blood cholesterol mg./%	Blood cholesterol esters mg./%	Blood cholesterol esters %	Plasma proteins gm./%	Hemo-globin %	Weight kg.
Mar. 15	114	55	1908	20.0	92	65	71	5.00	116	...
First Injection of Acacia (30 gm.) March 15th										
16	70	53	1431	15.6	79	54	68	3.50	...	15.3
17	82	73	1798	16.7	75	52	70	3.56	...	...
18	80	15	2037	15.4	65	45	69	3.69	...	...
19	84	56	1982	17.5	73	52	71	3.75	...	...
Third Injection of Acacia (20 gm.) March 29th										
29	122	53	1982	16.7	50	36	72	3.31	120	...
30	90	55	1706	15.3	49	37	76	2.42	...	...
31	92	55	1945	17.7	46	32	70	2.63	...	15.6
Apr. 1	100	59	2092	17.9	36	25	70	2.38	...	...
2	102	51	2055	19.2	34	24	71	2.56	...	...

30 gm. acacia injected on March 15th, 23rd and 29th, and April 10th. Total 120 gm.

percentage of circulating plasma proteins and this lower level was maintained. After the 3rd injection there is again a slight decrease in the bile salt excreted on the following day and there is further reduction in the plasma protein. It is of interest that no edema ever developed, even though the plasma protein level was much below 4 per cent. The acacia that remained in the blood compensated for the reduced plasma proteins and prevented formation of edema.

The total blood cholesterol is now at a lower level than previously but the ratio of esters of cholesterol to total is maintained at the normal level of 70 per cent. This is the effect of total deprivation of bile as mentioned previously.

In Table IV the results of feeding bile salts are tabulated. Four samples of dog bile (75 cc.) were given by stomach tube on succeeding days. These portions of bile contained 1219 mg., 1402 mg., 1456 mg. and 1454 mg. of bile salt, respectively, or a total of 5531 mg. Over a 10 day control period the bile salt excretion averaged 2027 mg. daily and during the experimental period 4773 mg. were excreted in excess, or 86 per cent recovery of the amount fed. The bile cholesterol is increased also as the result of the extra salt in the bile, but the blood cholesterol is not altered. The plasma protein continues at the low level consequent to the previous acacia injections.

The dog was next starved for 1 day and then chloroform (5 cc.) was given by stomach tube on the following 2 days. There is immediate decrease in the bile volume with the bile salt content reduced and it is only after 6 days that the bile salts return to normal. The bile cholesterol is reduced in amount but the blood cholesterol is not affected, and the bile volume returns to normal with increase in the amount of bile pigment eliminated. The plasma proteins still are low. Ten days after this period carbon tetrachloride (5 cc.) was given by stomach tube. The bile salts decreased from 1541 mg. to 917 mg. following this 1st dose. The dog was less active on this day but did not appear ill and another 5 cc. dose was given. The next morning the dog was found dead as the result of severe hemorrhage into the peritoneal cavity. The plasma proteins were at the low level of 2.9 gm. per cent and the fibrinogen would be greatly reduced, as illustrated by data on similar dogs.<sup>7</sup> Another factor that may well be responsible for the



TABLE IV  
Effects of Bile Salt and Chloroform Feeding on the Acacia Filled Liver

Dog 37-106

Date	Bile volume cc.	Bile pigments mg.	Bile salts mg.	Bile cholesterol mg.	Total blood cholesterol mg./%	Blood cholesterol esters mg./%	Blood cholesterol esters %	Plasma proteins gm./%	Hemo-globin %	Weight kg.
Bile by Mouth (75 cc.) April 4th, 5th, 6th and 7th										
Apr. 4	110	53	1885	19.6	40	25	63	2.69	...	...
5	170	66	2844	23.9	40	28	70	2.75	...	...
6	184	63	3174	23.6	44	31	70	2.94	114	...
7	212	63	3633	26.2	48	33	69	3.00	...	15.7
8	198	71	3230	26.0	46	32	70	3.06	...	...
9	150	59	2183	17.6	44	26	59	3.12	...	...
Chloroform (5 cc.) by Mouth April 13th and 14th										
12	70	64	1559	11.1	35	25	71	2.23	90	15.7
13	70	95	1541	14.0	38	28	73	2.44	...	...
14	34	48	734	5.0	35	23	66	2.19	...	...
15	5	..	624	...	35	19	54	2.25	...	...
16	24	61	404	2.0	39	25	54	2.06	...	...
17	60	66	624	...	...	...	..	...	...	...
18	44	94	697	3.9	37	23	62	2.75	...	...
19	70	110	1028	7.1	30	21	70	2.75	101	...
20	94	120	1358	11.1	30	20	71	2.81	...	15.5

spontaneous hemorrhage is the low prothrombin resulting from lack of bile in the intestine. Chloroform and carbon tetrachloride causing injury to the liver may in themselves lower the fibrinogen and prothrombin, so it is not surprising that spontaneous hemorrhage occurred.

At autopsy the peritoneal cavity was filled with unclotted blood but no definite bleeding point could be established. The pleural and pericardial sacs were normal. The heart and lungs were normal grossly and histologically. The blood within the heart was not clotted and no clot formed on standing. The liver was nearly twice the normal size and was friable and reddish in color. Its lobules were indistinct. The cannula was *in situ* in the common bile duct and the ducts all appeared normal. The gastro-intestinal tract contained some granular blood stained material in the terminal ileum but no bleeding points were found. Other organs appeared normal.

Histologically the liver shows extreme necrosis as only a few viable cells remain about the portal areas. These cells are vacuolated and reticulated in appearance due to the acacia. The rest of the lobule shows hyaline necrosis with cells indistinct and with no nuclei. Red cells are numerous in the necrotic areas. Sections of liver stained with sudan IV show no visible fat present.

### DISCUSSION

It is apparent that in bile fistula dogs the plasma proteins are markedly reduced in amount following repeated intravenous injections of acacia. The livers of such dogs are increased in size due to the accumulation of the acacia within the liver cells. Heckel and co-workers<sup>7</sup> have shown that with the decrease in plasma proteins there is particularly a concomitant much greater reduction in the fibrinogen. It is to be admitted that the plasma proteins may be decreased to compensate for the acacia which continues to circulate in the blood stream. However, the fact that the reduction in fibrinogen is marked and not in proportion with the decrease in total plasma proteins suggests strongly the possibility that the acacia within the liver cells is interfering with their function of producing fibrinogen. The data obtained from these fistula dogs indicate that the presence of the acacia does not interfere with the activity of the cells in forming bile salts or in eliminating bile pig-

ments. Furthermore, the normal relation between the total cholesterol and esters of cholesterol in the blood is maintained and this might well be expected to be altered if the acacia were causing any serious injury to the cells. The total cholesterol does decrease but the ester percentage remains in the normal range. This is the result of the total bile deprivation causing disturbances in absorption of fatty materials from the intestinal tract. When bile salts are fed they are absorbed and the liver cells immediately excrete them into the bile, and from our data it appears that acacia does not interfere in this enterohepatic cycle of bile salt metabolism.

Andersch and Gibson <sup>6</sup> previously reported that the bile salt and bile pigments were reduced in amount following the intravenous injection of acacia. They describe a bile that loses its normal pigmented color. This is similar in character to the fluid that was excreted by one of our dogs when the fistula became obstructed. We have repeatedly observed such bile in other fistula dogs whose fistula has become obstructed or infected. In view of these facts we feel their data regarding bile salts and bile pigment are not significant but the other data relating to the removal of acacia from the blood stream by the liver cells and its elimination in the bile are correct.

One constant finding in our experiments is that on the day following the injection of the acacia there was a decrease in the bile salts excreted. There is no prolonged reduction and therefore it does not seem that this decrease is due to the presence of the acacia in the liver cells. It is possible, however, that immediately following the injection the functions of the liver cells are temporarily slightly deranged due to the intrusion of the acacia. Another factor in this 1 day decrease in bile salt excretion may be the vomiting that occurred immediately following the injection of the acacia. In other fistula dogs we have observed that vomiting may affect the amount of bile salt excreted.

Since fibrinogen production is affected by the acacia in the liver cells without impairment of their ability to form bile salts, we have still another instance of dissociation of the functions of the liver cells. It might be well to mention a few other examples of such dissociation. A mild liver injury may greatly reduce the bile salt production but the volume of bile and the amount of bile pigments eliminated may remain normal. Likewise, bile salts <sup>2</sup> and pro-

thrombin<sup>18</sup> may be low but the fibrinogen remains normal when injury is not severe. In bile fistula dogs<sup>19,20</sup> the fibrinogen may be normal and yet the prothrombin may be so decreased in amount that spontaneous bleeding occurs. Inflammation<sup>21</sup> anywhere in the body may cause great elevation in the fibrinogen with no change in the prothrombin. Since the liver has such a wide variety of functions and since there is very frequently this dissociation, it is not surprising that no adequate test of liver function has been established.

### SUMMARY

The repeated intravenous injection of acacia in bile fistula dogs results in enlargement of the liver due to the accumulation of acacia in the liver cells. The plasma proteins progressively decrease and are maintained at a level much below normal as a result of the acacia within the blood stream and the liver.

Acacia within the liver cells does not seriously interfere with the cell's ability to form bile salts or eliminate bile pigments, and it does not disturb the enterohepatic cycle of bile salt metabolism when bile salts are fed. The fed bile salt is absorbed and excreted promptly into the bile.

Bile and blood cholesterol metabolism are not altered as the relation between the total blood cholesterol and esters of cholesterol is maintained within the normal range. In the bile fistula dog the total cholesterol of the blood decreases, but this is related to faulty absorption of fats due to total bile deprivation.

Chloroform and carbon tetrachloride when given by mouth cause injury to the liver with reduction in bile salt formation. The injury in one instance was severe enough to cause spontaneous bleeding with death from hemorrhage, indicating interference with formation of fibrinogen and prothrombin.

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## MOOSE ENCEPHALITIS \*

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A disease of obscure nature affecting moose was referred to in the literature by Cameron and Fulton<sup>1</sup> in 1926, but practically all that is definitely known about the condition has been contributed by Fenstermacher and Jellison,<sup>2</sup> Fenstermacher,<sup>3,4</sup> and by Thomas and Cahn and coworkers.<sup>5-8</sup>

The diseased animals when first observed show but little of their customary fear of man. They may be fairly readily approached and may sometimes even be led into captivity. Weakness and unsteadiness, with a tendency to staggering gait but without true paralysis, are common. Less frequently there may be signs of impaired vision or peculiar attitudes of the head. Emaciation is observed in the majority of animals but this is by no means constant.

The condition does not show any definite seasonal incidence. Of 23 cases reported from Minnesota<sup>2,3,4</sup> the distribution by months was as follows: April, 6; March, 4; October, 3; January, February and May, 2 each; June, July, September and December, 1 each. In Maine † the incidence of 20 cases was: March and April, 4 each; February, 3; May and June, 2 each; and January, July, August, October and December, 1 each. The majority of the cases have thus occurred in the months from February to May, but every month except November has had at least 1 case.

In pathological observations (sometimes incomplete) of 23 animals<sup>2,3,4</sup> a variety of parasites was observed in the lungs, liver, intestine, heart and the eye in different animals. Most of the animals showed infestation with the tick *Dermacentor albipictus*, frequently to an extremely severe and extensive degree. However, a rare animal may show no ticks at all and others show only minor degrees of infestation. Many of the moose showed a secondary

\* An opportunity to study this disease was presented through the cooperation of Prof. E. C. Nelson of the University of Maine, and of Dr. F. Fenstermacher of the University of Minnesota.

† Personal communication from Prof. C. M. Aldous and Mr. A. L. Lamson.

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type of anemia with basophilic stippling in the erythrocytes. In 2 cases evidence of inflammatory reaction was found in the brain. In 1 of these 2 animals an unidentified nematode was also found in the brain but without attending reaction. The inflammation occurring elsewhere in the brain was probably independent of this parasite. In 4 additional brains only small areas of hemorrhage were found. Apart from these observations in the brain, pathological features that might be relevant to the disease picture were not noted.

Attempts at autopsy to recover pathogenic infectious agents were uniformly unsuccessful, not only by ordinary bacteriological culture, but also by intracerebral or other inoculation of tissue suspensions, including that of the brain.<sup>2,3,4</sup> On the other hand, Thomas and Cahn and associates<sup>5-8</sup> studied ticks infesting pieces of moose hide cut from dead animals and shipped to Illinois. When guinea pigs were infested with these insects a fatal disease was produced supposedly similar to what had been described in the moose. From the ticks a new bacterium was isolated, *Klebsiella paralytica*, whose properties have been carefully described<sup>8</sup> and which was suggested as the cause of the disease in moose. However, Fenstermacher, working directly with moose as soon as they were killed, has been unable to confirm these observations. A bacterial origin for "moose disease" cannot be considered as established.

A typical example of the disease was observed at the University of Maine, with the following history.\*

On October 21, 1938, a moose was reported wandering around on the highways by the wardens near West Rockport, Maine. It seemed fairly strong but was thin and very tame. It had been seen for 3 days when reported. The animal was loaded into a truck and brought to Orono and placed in about an acre sized pen in the woods. In the pen it showed a good appetite and browsed; it was also fed carrots, cod liver oil and bone meal. On November 10th marked drowsiness, drooping of the hind quarters and a tendency to fall developed. On November 13th the animal fell in a brush pile and was able to get up only with help. A peculiar lopping and a flick of the right ear were noticed, as well as an inter-

\* Gathered by Mr. Lamson and Dr. Witte, and kindly supplied to the author by Prof. E. C. Nelson.



mittent facial twitch on the right side. On November 14th the animal went down and could not get up even with help. A pronounced right twist of the neck was noted the next day. On November 16th the moose lay flat on its left side with the neck extended. Marked edema of the eyelids was present and the eye on the left side was clouded and apparently blind. The animal was shot through the heart and autopsied immediately.

The head was removed at autopsy, packed in solid carbon dioxide and shipped to this laboratory, where it arrived on the 2nd day following. The tissues were in an excellent state of preservation, considering the lapse of time since death. On removal of the brain no gross abnormalities were observed. The nose, sinuses and ears were entirely normal. The edema of the eyelids mentioned in the history was not observable after the lapse of time involved during shipment, and the eyeballs showed no abnormalities. Some of the brain material was saved for passage and the remainder fixed in 10 per cent formalin or in Zenker's fluid. This animal is designated hereafter as Moose 1.

For further study the following additional material was obtained from Prof. Nelson: portions of a brain and cord of a 2nd moose fixed in 10 per cent formalin for 11 months, and an entire brain of a 3rd animal, fixed in formalin for 8 months. Dr. Fenstermacher kindly furnished paraffin blocks of parts of the brain from 5 additional animals which had been autopsied in Minnesota. Material from 8 animals was thus studied.

### OBSERVATIONS

The following description is drawn chiefly from Moose 1 whose history has been detailed above. Reference to the other cases is made where indicated.

In the brain several different types of pathological change may be observed, the interrelations of which it is not always easy to determine. There is a definite loss of myelin, although of an unusual type. In frozen sections stained with scarlet red considerable quantities of neutral fat may be observed in scattered parts of the white matter. In Figure 1 is seen a low power view of a portion of the corpus callosum illustrating an unusually severe degree of fat formation. The fat stains a brick red, rather dull in color, without the brilliance frequently seen in other demyelinating

conditions. Under polarized light there is no double refraction. The lipoid droplets do not stain with hematoxylin in myelin stains. That all the fat is intracellular, within phagocytes, cannot be satisfactorily shown. Stains of cells in paraffin embedded tissues show very few typical compound granular corpuscles. This may be due to cytoplasmic disintegration in the period between death and fixation of tissue, or it may be due to the absence of such cells. That neutral fat can occur in the brain in the absence of phagocytic or other cellular action is not surprising, for this type of fat formation has previously been demonstrated in multiple sclerosis.<sup>9</sup> Similar, though less intense areas of fat formation have been found in the centrum ovale and in the white matter of the cerebral convolutions. In such regions, as in Figure 1, the lipoid droplets are scattered more or less uniformly in very poorly defined foci. Sharply circumscribed areas showing loss of myelin, such as are seen in multiple sclerosis, do not occur. Where the free fat is scattered in the tissue the myelin sheaths may be moderately diminished in number but are not totally lost.

The presence of diffusely scattered fat is indicative of an acute process. In other portions of the brain there is an accumulation of lipoid only in the perivascular spaces, but none in the intervascular areas. Such a field is illustrated in Figure 2 and is a sign of an older process than that shown in Figure 1. The total amount of fat observed in the entire brain, both in the parenchyma and in the perivascular spaces, is not large.

In other regions, where there is no sign of recent destruction, many scarred areas demonstrable with stains for glial fibrils are found. These scars are present in the medulla, centrum ovale and convolutional white matter of the cerebrum, and also in the white matter of the cerebellum. In Figure 3 is seen such a scar in the medulla. The proliferation of glial fibrils is quite intense but the actual increase in astrocytes, *i.e.* in the number of cell bodies, is not great. The occurrence of intense fiber proliferation, in the absence of significant cellular increase or of progressively altered cell forms, shows that the repair process is completed. The original insult occurred probably some months previously.

Two types of glial scars are illustrated in Figures 4 and 5. Figure 4 is from the centrum ovale of Moose 1 and shows several small perivascular scars. Figure 5, from the cerebellum of Moose

6, shows a dense isomorphous glial feltwork, diffuse rather than perivascular. Here the process is quite old, for well defined astrocytes are rare, although the fiber proliferation is great. On the other hand, in Figure 6, from Moose 1, astroblastic forms are clearly seen among the glial fibers.

The loss of myelin is generally mild, invariably perivascular, and practically always much less in extent than is the fibrous gliosis. In Figure 7, from Moose 1, the destruction of myelin, although slight in absolute terms, is very severe as compared with that in other parts of the brain. In Figure 8 is seen a section, adjacent to that shown in Figure 3, of the medullary reticular formation but stained for myelin. Considering the thinness of the section ( $12\ \mu$ ) it is readily seen that the loss of myelin is disproportionately small compared with the density of the gliosis (Fig. 3). And in the myelin stained section adjacent to that of Figure 5 no loss of myelin at all can be discerned in the corresponding area. In the section adjacent to that of Figure 4, but appropriately stained for myelin, there are small clear areas surrounding the affected blood vessels.

Axis cylinders are somewhat better preserved than the myelin sheaths but not to any marked degree. But, as has been emphasized elsewhere,<sup>10</sup> in the loss of myelin from whatever cause the axis cylinders are always less affected than the myelin sheaths.

There is abundant cellular reaction, chiefly perivascular. In Figure 2, for example, the cells in the blood vessel sheaths are chiefly concerned with the phagocytosis of lipoids. Elsewhere, however, as shown in Figure 9, there may be a true inflammatory reaction with abundance of lymphocytes around the blood vessels and even some diffuse tissue infiltration. Polymorphonuclear leukocytes were not seen. This inflammation may be of the "secondary" or symptomatic type for, as is well known, it is frequently seen in various non-infectious demyelinating diseases. In the sections corresponding to Figure 9, but stained for glia and myelin, a fibrous gliosis could not be demonstrated. Around the more severely involved blood vessels was a mild degree of loss of myelin.

With one single exception, the inflammatory reaction, where present, was restricted to the white matter. In the exceptional instance (Moose 5 of this series) the gray matter was affected.

This occurred in 1 case sent by Dr. Fenstermacher where, slightly involving the entorhinal cortex, there was a reaction very similar to what he has illustrated in his Figure 3.<sup>3</sup> Moose 5 of this series is the same animal from which Dr. Fenstermacher's photograph was taken.

The neocortex was intact in all available material. For the most part the tissue was not sufficiently well preserved for cytological examination. Most of the ganglion cells showed severe swelling, vacuolation, and other postmortem artefacts. But the architectonics appeared normal and no areas of loss of cells, inflammation, or of meningitis were observed.

Of the 8 cases available for study, 3 came from Maine, of which 2 showed similar pathological lesions, while in the 3rd no lesions of any sort could be found. This last case, however, stained poorly. Of the 5 cases, blocks from which were sent from Minnesota by Dr. Fenstermacher, gliosis with more or less demyelination was found in 2. In a 3rd, of which only a few blocks were available, the only pathological change observed was the inflammatory reaction in the gray matter referred to above. In the remaining 2 cases no abnormalities could be detected. In 1 of these 2, however, death was probably due to distomiasis and not to moose encephalitis (personal communication from Dr. Fenstermacher; material to be published). Thus, in 7 probable cases of the disease in question, characteristic changes were observed in 4, while 1 other appeared atypical. In reference to the negative findings it should be pointed out that all sick (or dead) moose, even with fairly similar symptoms, are not necessarily affected by a single disease. Fenstermacher<sup>2</sup> is strongly of the opinion that "the losses of moose that occur in Minnesota are not the result of a single pathogen."

From the whole brain that was received unfixed from Maine, portions were emulsified for animal passage. Sheep, kittens, mice and a pig were inoculated. One sheep died of bacterial meningitis, but a 2nd animal survived without symptoms. All the other injected animals also showed no symptoms. These results agree with Fenstermacher's inability to reproduce the disease by inoculation.

## DISCUSSION

The occurrence of neutral fat, perivascular areas of demyelination, gliosis, and moderate inflammatory reaction in the brains of moose raises the question of a possible relation to multiple sclerosis and other demyelinating diseases of man and animals. One chief difference from multiple sclerosis is that in the latter disease the areas showing loss of myelin, although frequently perivascular in early lesions, usually develop to have no relation to blood vessels.<sup>11</sup>

In the moose, the loss of myelin is disproportionately small compared with the extent of the gliosis, somewhat reminiscent of the human cases reported by Müller<sup>12</sup> and by Bodechtel and Guttmann,<sup>13, 14</sup> and not at all similar to multiple sclerosis. It is necessary to agree with their statement that gliosis is not merely a defect filler but may be induced independently. In a previous communication<sup>9</sup> it was pointed out that the gliosis which occurs in multiple sclerosis cannot be considered as merely secondary to the loss of myelin. This statement must be repeated in relation to the disease in moose.

It is well known that in the central nervous system there is no necessary connection between inflammation and loss of myelin. Glial proliferation may occur as a result of an inflammatory reaction in which myelin has not been significantly destroyed. Yet in the disease in moose the histological picture does not suggest a primary inflammatory condition such as is found in many virus diseases. An exception to this statement is Moose 5, referred to above, with a typical primary inflammatory reaction involving the gray matter, a condition which does not fit in with other cases of the series. Although in this single case only a few blocks were available for examination, the evidence is strongly suggestive that this one instance may represent a quite different disease entity. In the 4 other positive cases the changes observed were strictly those of a leukoencephalitis. Moose 5 was not in this category.

For the present, until further data become available, this leukoencephalitis must be considered as a disease entity in moose, with the strong possibility that there may also be another form of encephalitis.

It must be emphasized that the disease process as disclosed by the present study is evidently a subacute or chronic condition.

The glial scars are at least of several months duration, and not improbably even older. At the same time activity of the disease shortly before death is shown by the occurrence of neutral fat. The immediate cause of death, however, as in most neurological conditions, is not apparent.

The etiology of the demyelinating condition remains obscure. Attempts by Fenstermacher and by ourselves to transmit the condition by tissue inoculation have been negative. This failure is not a cogent argument against an infectious etiology, but the evidence is supported by the fact that none of the many forms of leukoencephalitis has ever been shown to be caused by an infectious agent. That the bacterium *Klebsiella paralytica* is the causative agent must remain questionable until confirmed. Attempted confirmation has not proved successful.<sup>3</sup>

The role of the tick infestation remains equally obscure. The condition of tick paralysis, in animals and man, is well recognized as a naturally occurring disease and has been reproduced experimentally.<sup>15, 16</sup> However, there are no adequate studies of the pathology of this condition, and the clinical course does not suggest a kinship. A toxic factor resulting from the tick infestation cannot be arbitrarily ruled out.

Thrombosis and vascular occlusions as the cause of demyelinating lesions have been claimed by Putnam.<sup>17</sup> In the present instances evidence of thrombosis was not observed.

There is no evidence throwing satisfactory light on the etiology of this disease. Assuming that the great majority of dead or sick moose observed are suffering from a single disease entity, and considering the total moose population of Maine and Minnesota, the incidence of this disease is high, suggesting either an infection or a common environmental factor such as a dietary deficiency or a toxic substance. Grounds for a decision are not as yet available.

### SUMMARY

A subacute or chronic leukoencephalitis occurring naturally in moose is described. The characteristic picture consists of a mild degree of perivascular demyelination, with formation of neutral fat, and with fibrous gliosis disproportionate in extent to the loss of myelin. There may be mild inflammation restricted to the white matter. There is suggestive evidence that a primary inflammatory

reaction involving gray matter and observed in 1 animal out of 8 may represent a separate condition. Attempted animal passage of fresh material from 1 case was unsuccessful. The etiology of this leukoencephalitis is obscure although various possibilities are discussed.

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## DESCRIPTION OF PLATES

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### PLATE 74

- FIG. 1. Cerebral white matter, stained for fat. The stainable lipoids are diffusely scattered throughout the affected focus.  $\times 50$ .
- FIG. 2. A different field from the same animal. The neutral fat is located almost exclusively in phagocytes in the perivascular spaces. The insignificant loss of myelin can be readily appreciated even with the fat stain.  $\times 133$ .
- FIG. 3. Reticular formation of the medulla oblongata, stained for glial fibers with Victoria blue. Dense, moderately well circumscribed perivascular gliosis is present. This figure should be compared with Fig. 8, the same field of an adjacent section stained for myelin.  $\times 105$ .
- FIG. 4. Centrum ovale, stained for glial fibers. The small scars are strictly perivascular.  $\times 47.5$ .



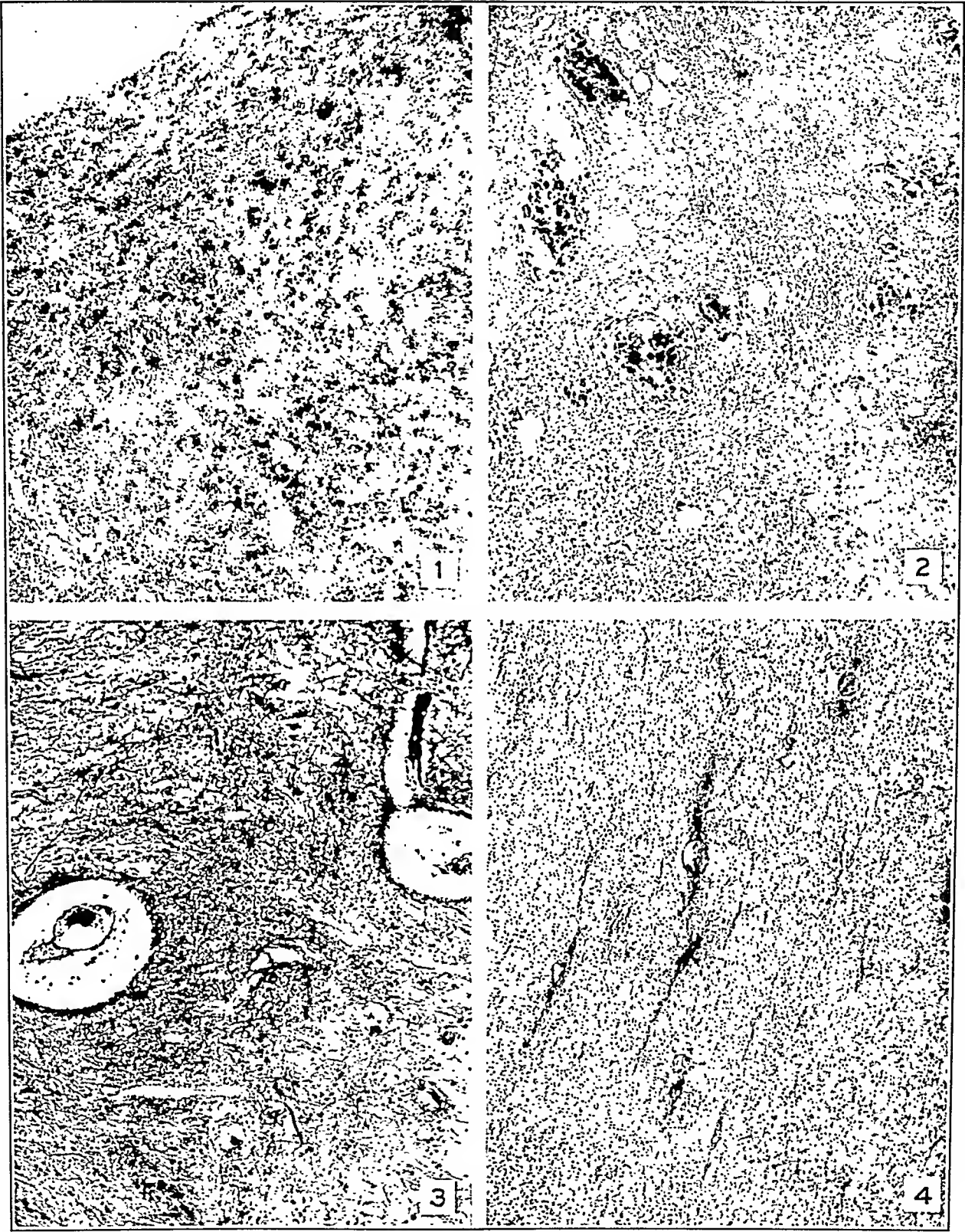
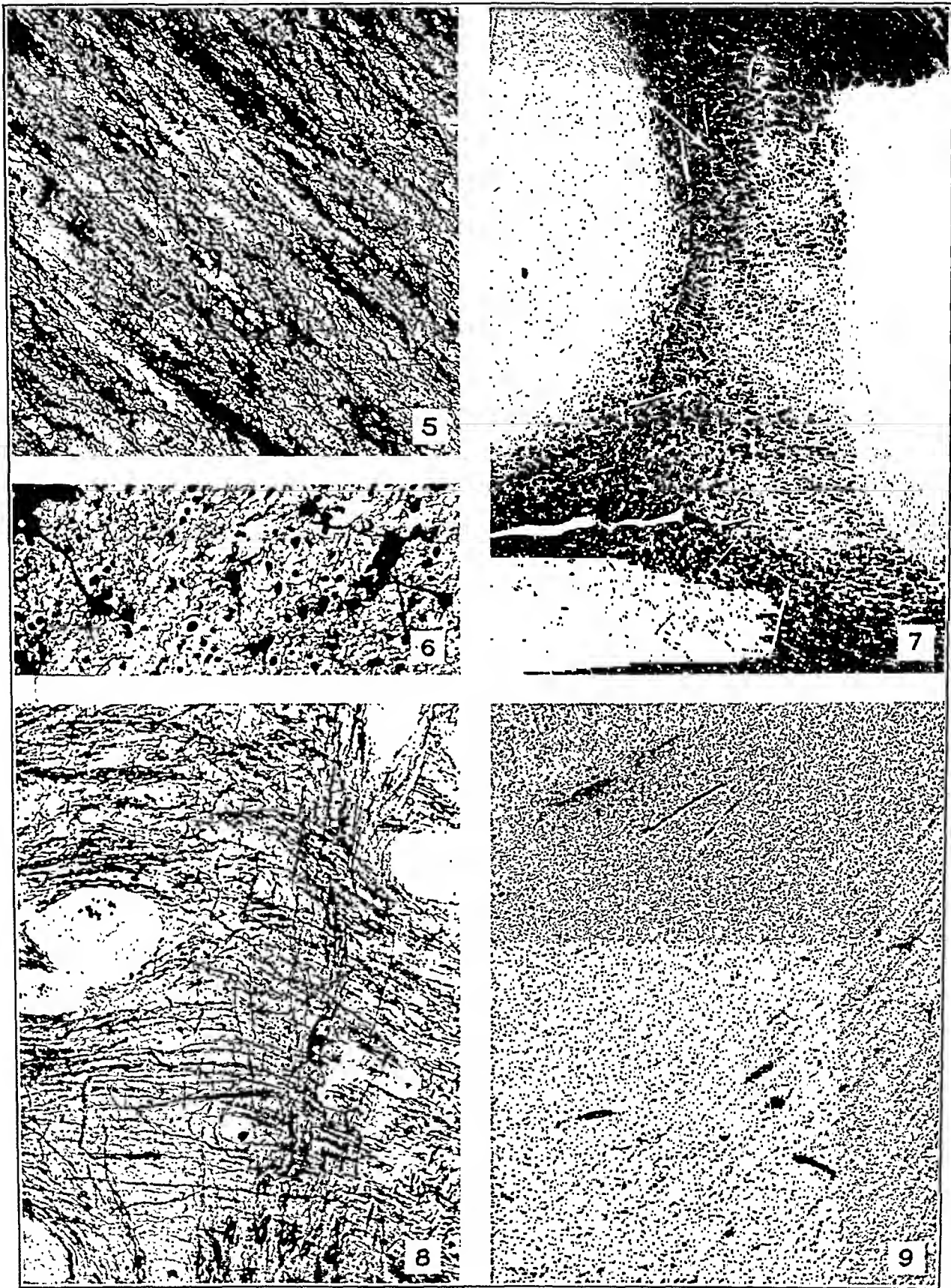


PLATE 75

- FIG. 5. Cerebellar white matter, stained for glial fibers. There is a very dense glial feltwork. Adjacent sections stained for myelin show no loss of myelin.  $\times 180$ .
- FIG. 6. From the centrum ovale, showing astroblastic forms in the midst of a mild diffuse gliosis.  $\times 230$ .
- FIG. 7. Cerebral white matter, stained for myelin. A slight degree of demyelination is evident around the smaller blood vessels.  $\times 18.6$ .
- FIG. 8. Reticular formation of medulla oblongata, stained for myelin. Fig. 3 shows the dense glial scar in this region. Fig. 8 illustrates the insignificant degree of loss of myelin.  $\times 105$ .
- FIG. 9. Centrum ovale. Inflammatory reaction in the white matter, not invading the cortex, visible at upper left. Toluidine blue stain.  $\times 35$ .





# THE DIFFERENTIATION BETWEEN SPIROCHETES AND SPIROCHETE-LIKE STRUCTURES IN THE PLACENTA \*

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## INTRODUCTION

The paucity of literature concerning the presence of spirochetes in the human placenta indicates that they have been observed in this location on but few occasions, and furthermore tends to raise the question of the validity of these observations.

Montgomery <sup>1,2</sup> has expressed the opinion that the discovery of *Treponemata pallida* in the placenta occurs so infrequently as to be of negligible value, and that while the human placenta may transmit the organism of syphilis from an infected mother to the embryo, it seems to possess "a peculiar resistance to the development of true syphilitic lesions" in itself. McCord <sup>3</sup> found no definite histological appearance of the placenta characteristic of syphilis. Routh, <sup>4,5</sup> in support of an earlier suggestion, states that "certain placental ferments" exhibit treponemicidal activity. Harrison <sup>6</sup> states that spirochetes have not been found frequently in syphilitic placentas but attributes this to the lengthy search required. Kaufmann, <sup>7</sup> however, states: "By the use of Levaditi's silver method, Paaschen, Wallich and Levaditi and many others have found spirochetes particularly in the villi; Mohn, *e.g.*, found them in 50% of the cases in the umbilical cord, and in the placenta, in 70% of the cases of syphilis of the parents."

In a previous paper Dorman and Sahyoun <sup>8</sup> reported the discovery of spirochetes in 105 placentas and outlined the method of examination. The confirmation of these observations by others would contribute materially to the elucidation of the pathogenesis of syphilis, and, for this reason, the full details of the methods used are being included in this paper.

As our methods include silver impregnation, it is worthy of mention that the interpretation of structures so impregnated is prone to be subject to controversy, although there is no more basis

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for general criticism of silver impregnation methods than there is for most staining methods, a view supported by the experimental work of Bofill-Deulofeu,<sup>9</sup> and of Robinow.<sup>10</sup> In the present study, relative to the validity of identification of silver impregnated, spirochete-like structures in placental tissue, we have: (1) applied methods designed to preclude or confirm the alternative possibilities that the observed structures might be fibrinous filaments, collagenous fibers, reticular fibers, mycelia, or other structures which are known to be subject to impregnation with silver after treatment with formalin; and (2) searched for positive evidence that the observed structures are in fact spirochetes.

### MATERIAL

The placentas examined were chosen by the clinician and were from patients with a positive serum reaction, or with a history of stillbirth, prematurity, or abortion, *i.e.*, from cases that were clinically suspected to be syphilis.<sup>8</sup>

Tissue from a chancre experimentally produced in the scrotum of a rabbit by inoculation with *Treponemata pallida* was used as a control for each of the technical procedures used.

### METHODS

The fresh placentas were placed in flat jars containing a 10 per cent neutral formalin solution for 24 hours. Ribbons of placental tissue about 0.5 cm. in thickness were obtained by slicing the partially fixed placenta in its longest diameter. Pieces of these ribbons 4 cm. in length were fixed in 5 per cent formalin for a minimum period of 3 weeks. Each piece was then halved by cutting through its most suspicious site and the resulting halves separated into two groups as follows:

*Group A:* This consisted of one block of each pair which was dehydrated, cleared and embedded in paraffin.

*Group B:* The analogous block of each pair in this group was impregnated with silver according to Nyka's modification of the Levaditi method,<sup>11</sup> and then dehydrated, cleared and embedded in paraffin.

Six corresponding sections varying in thickness from 5 to 15  $\mu$  were cut from each of the twin blocks and each section mounted on a separate slide. Of the 6 sections from each block of Group A,

2 were stained with hematoxylin and eosin, 2 with Weigert's safranelin and hematoxylin, and 2 with Mallory's aniline blue stain.<sup>12</sup> Of the 6 sections from each block of Group B (silver impregnated), 2 were deparaffinized, cleared, and mounted in Canada balsam; 2 were deparaffinized, counterstained with Mallory's aniline blue stain, cleared, and mounted in Canada balsam; and 2 were deparaffinized, washed in distilled water, treated overnight with a 1 per cent solution of potassium iodide, washed gently with bidistilled water, treated with a 5 per cent solution of sodium hypsulphite, washed in distilled water, dehydrated, cleared and mounted in Canada balsam. It was found that the handling of these slides after treatment with potassium iodide had to be done with care as the sections showed a tendency to be displaced easily. The purpose of this procedure was to obtain increased definition of a few of the spirochete-like structures, even though the silver precipitate was removed to such an extent that the number visible was greatly reduced.

#### MICROSCOPIC EXAMINATION

Sections from blocks of Group A served the purpose of ascertaining the general architecture of the placenta and of determining the topographical distribution of the pathological lesions. Particular note was taken of hyalinization, infarction, calcification, leukocytic infiltration, and the condition of the blood vessels, recording the changes in the latter as (*a*) no special pathology, (*b*) thickening of the walls with or without occlusion, and (*c*) periarteritis and endarteritis. More than one of these conditions was frequently found in a single section.

Sections from blocks of Group B (silver impregnated) were routinely examined in the following order: (1) ordinary silver impregnated sections; (2) silver impregnated sections counterstained with Mallory's aniline blue stain; and (3) silver impregnated sections treated with potassium iodide.

#### *Observations*

4. The ordinary silver impregnated sections are first examined with low power for certain small argentophilic foci. When present, such foci are usually found near the amniotic surface and occasionally on the decidual surface or in the region of the large blood

vessels. These foci are round, oval or serpiginous in shape. In some instances they are large enough to be seen without the aid of a lens. A number of these foci, marked with India ink, are then examined with higher magnifications. Under the oil immersion lens such foci are seen to contain numerous silver impregnated granules and spirochete-like structures. Usually the granules are more numerous in the peripheral zone, and in some instances they are so concentrated in the periphery as to demarcate the focus with a dark ring. The spirochete-like structures are arranged singly or in groups; sometimes two or more are intertwined and appear as a thick cord, or two or three may be intertwined end to end. Study of the above conditions was facilitated by use of a binocular microscope equipped for stereoscopic vision. By this means structures appearing as a tangled mass of black lines can be distinguished from one another as they arise into perspective; their coils are emphasized and the individual filamentous components are optically distinguishable, appearing in their homogeneous background as distinctly discrete, spirally coiled bodies.

*B.* A counterstain of the silver impregnated sections with Mallory's aniline blue stain was done to differentiate between the collagenous fibers and the spirochete-like structures. Staining with Mallory's aniline blue method following impregnation with silver gives a degree of selective staining analogous to the selective affinities shown by Mallory's stain after Zenker fixation, but not otherwise obtained in formalin-fixed tissues. The staining affinities of the different components of the placental substance from the amniotic surface outward, including the foci, are as follows:

1. The cuboidal cells of the amniotic membrane take a brownish golden color, contrasting with the blue-staining, delicate collagenous fibers which extend from the underlying layer and surround each cell.

2. A thick, deeply stained layer of blue collagenous fibers forms the second layer, from the inner surface of which delicate fibers extend to the amniotic membrane, and from the outer surface of which grow the chorionic villi. This second layer contains embedded in its substance both the large blood vessels and the scattered groups of Langhans' cells, the latter appearing as pale brownish, polyhedral cells with brownish yellow nuclei.



3. The chorionic villi, rich in the blue-staining collagenous fibers of the stroma, are covered by syncytium which is stained purplish violet. Delicate blue collagenous fibers extend from the stroma of the villi and enmesh the syncytial masses.

4. The smooth muscle cells of the blood vessels take a golden brown color and the erythrocytes a golden red.

5. The cells of the decidua take a brownish golden color, conspicuous in their blue environment.

6. Fibrin and fibrinoid filaments, as well as the amorphous hyaline, stain yellowish red.

7. Areas of calcification, when present, appear as amorphous black masses surrounded by a dark background.

8. Infarcted placental substance loses its property of staining in proportion to the age of the infarct. The most advanced infarcts are yellow and contain a few, irregularly distributed, grayish blue collagenous fibers.

9. The foci become yellowish red and appear to be related to the periarteritic and endarteritic blood vessels, as such damaged blood vessels are usually found in the neighborhood and in some instances in the center of these foci. Villi may be found in their vicinity and remnants of villi may even be within the focus, but these structures, owing to their blue collagenous content, stand out prominently in the yellowish red matrix of the focus. The black spirochete-like structures appear as definite forms showing distinct ends which have no connection whatsoever with contiguous blue-staining collagenous fibers or yellowish red, fibrinous filaments. No stage of transition is found between the spirochete-like structures and the blue collagenous fibers. In but few instances are these black structures seen among the blue-stained collagenous masses, but even here they stand out as sharply defined black objects which exhibit no relation to, continuity with, or transition from the blue collagenous fibers.

C. The silver impregnated sections treated with potassium iodide are then examined. The method of clearing the impregnated sections of excess silver ("desilverization," if it may be so called) is a new procedure which has been of material help in our study. The sections become very pale and require painstaking search and thorough study. The areas in which the foci should be found are marked with India ink before examination.

On examination with low magnification very little can be made out as the sections show a uniform, very faint brownish yellow color. With the oil immersion lens it is found that the black granules have lost their color completely and have merged into the background. The majority of the spirochete-like structures appear as almost colorless, transparent refractile filaments, but some have their silver coating sufficiently preserved so they can be sketched with a camera lucida (Text-Fig. 1). The increased sharpness in definition compensates for the decrease in number. The spirochetal structures which have been freed of excess silver appear as cylindrical spiral bodies 10 to 12  $\mu$  in length, 1  $\mu$  in thickness, and consist of 8 to 10 coils. The distance between the crests of the coils is from 1 to 1.25  $\mu$ . The coils are more open toward the ends of the bodies and the ends are tapering. As a result of the treatment with potassium iodide there is a slight increase in the diameter of these structures. This increase, however, amplifies the curvature of the coils and emphasizes their spiral nature.

Study of the syphilitic rabbit tissue which served as a control showed that the appearance of the foci, the location of the spirochetes and their relation to the argentophilic granules, and the staining affinity of the components of the foci were strictly analogous with those features as described in the placenta. Microphotographs would have been duplicates of those of the placenta (Figs. 1-3).

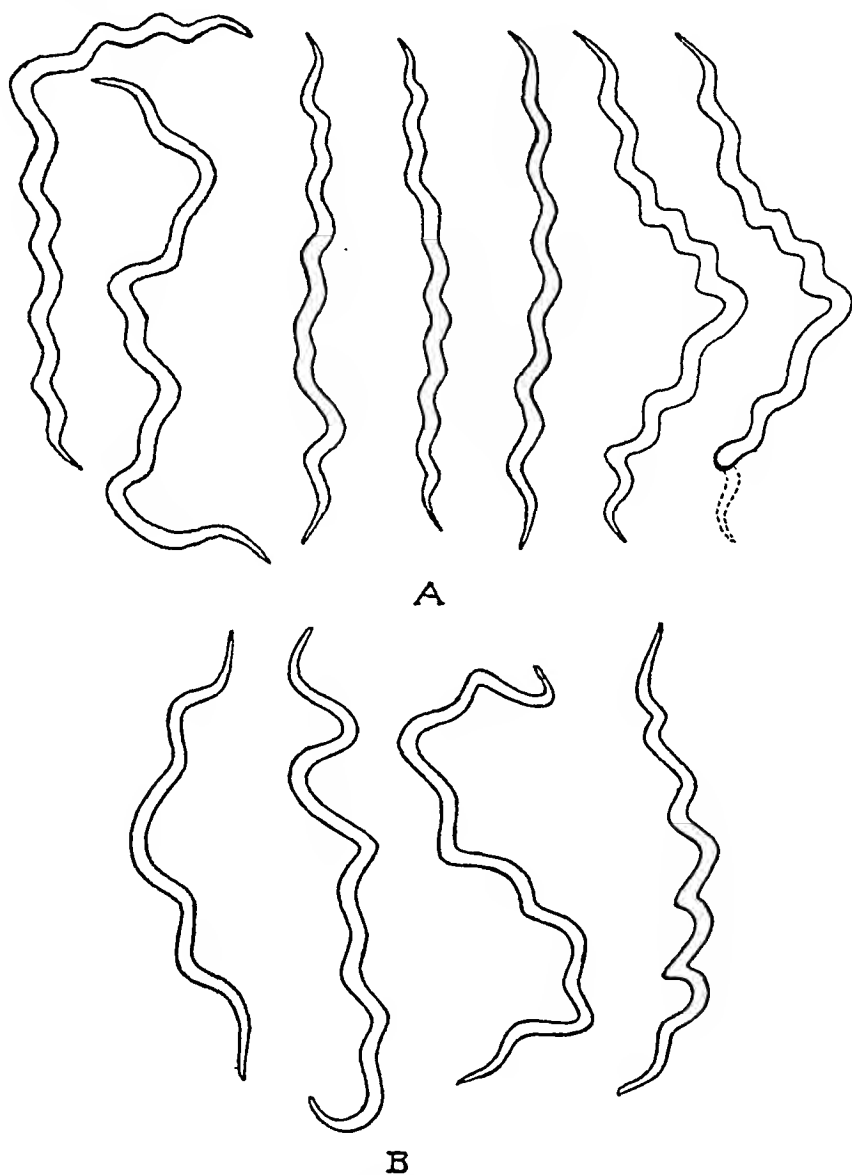
In connection with the above observations and the illustrations, it is important to realize the technical difficulties in the study and illustration of spirochetes in sections. In order to make the nature of these difficulties clear, they may be summed up as follows:

1. Complete spirals as such are not visible under high power, as only those segments of the spirals which happen to be in the plane of focus can be seen at any one time. These appear as interrupted segments of arcs which follow a certain rhythm depending on the size of the coils, their amplitude, and whether the spiral is straight or tortuous.

2. As the focus is changed, that plane of the spiral which was in focus disappears and is replaced by another plane which shows other segments of arcs. These segments follow a rhythm similar

to those in the former plane, but take another direction depending on the plane and the angle of vision.

3. When the spiral is of fine caliber, the segments that can be seen in focus are so small that they appear like beads, and it is, therefore, difficult to show complete spirals in one microphotographic field.



TEXT-FIG. 1. A. Semidiagrammatic camera lucida drawings of 7 spirochetes after removal of excess silver with potassium iodide. In No. 7, one of the ends has been reconstructed, as this end was lying in a plane perpendicular to the field of vision.

B. Semidiagrammatic camera lucida drawings of 4 *Treponemata pallida* from a syphilitic chancre of a rabbit.  $\times 4166$ .

4. In order to show the complete spiral by means of high power, the observer must focus constantly up and down while drawing. The resulting picture can, at best, be a mental one showing the way the mind of the observer has interpreted the shifting scene before his eyes.

In articles and monographs dealing with spirochetes in sections the value of the statements and microphotographs should not be judged without having in mind the above mentioned optical facts.

### DISCUSSION

Study of the hematoxylin and eosin-stained sections serves the purpose of ascertaining the general architecture of the placenta and of determining the topographical distribution of the pathological lesions. Such a study shows that periarteritis and endarteritis are the only findings that suggest the likelihood of finding "spirochetal" foci in the corresponding silver impregnated sections. It is of special interest to note that although the spirochete-like forms are found in the neighborhood, we are unable to find these structures in the occluded vessels themselves. Hyalinization has been seen in all of the placentas we have examined and is of no diagnostic value, even though the foci under discussion usually occur in such areas. Likewise, infarcts have no special significance for our problem as the argentophilic spirals have not been found in them, whether the infarcts be fresh or old. Calcification is so irregular in occurrence and distribution that it must be considered as irrelevant to the problem.

That the foci described in the silver impregnated sections are lesions is strongly suggested by their nature and contents, *i.e.*, they constitute abnormal entities which can be traced through the different sections of the same block, and it is hardly conceivable that accidental defects would be so uniformly consistent as to location, shape and contents.

Homma,<sup>13</sup> who recently studied silver impregnated sections of clinically syphilitic and normal placentas, described certain argentophilic structures found in all of his material, which, because of their size, shape, distribution and lack of relation to anything definable as a lesion, he rightly concluded were non-spirochetal in nature. However, the structures he described have no bearing on the argentophilic spirochete-like structures described in the present paper.

That the argentophilic spirals observed by us are not collagenous fibers is established by the following facts. The described foci, in the Mallory's aniline blue counterstained sections, are of a hyaline appearance, *i.e.*, they take a red stain and are readily distinguished from villi and remnants of villi which may be found in the neighborhood, as these are rich in collagen and as a result stain blue. For the same reason they can be distinguished from blood vessels whether they appear normal, or partially or completely occluded. The foci themselves do not contain collagenous fibers. Examination of these foci with the oil immersion lens shows the spirochete-like structures as definite black objects of uniform shape, clear-cut outline and sharp ends. In contrast, the collagenous fibers are blue and vary in shape, length and caliber; they may be wavy but are never spiralled. These distinguishing features eliminate the possibility of the black spirochete-like objects being collagenous fibers. Moreover, healthy collagenous fibers are never stained uniformly black with silver, and the black spirochete-like structures have not been found in any of the infarcts where degenerating fibers are to be expected.

That the argentophilic spirals are not reticular fibers is established by the following facts. Although the reticular fibers do stain black with silver, they show a definite relation to the collagenous fibers, with transitional stages. Reticular fibers are not uniform in length, thickness or coils. On the other hand, the spirochete-like structures are fairly uniform in shape, size and caliber and they show no relation to, transition from, or continuity with, the collagenous fibers found in the neighborhood of the focus. We considered the possibility of their being fragments of reticular fibers, but after comparing them with known fragments of reticular fibers, and for the reasons cited above, we could find no evidence to support this view.

That the argentophilic spirals are not fibrin or fibrinoid is also established by the following facts. In the counterstained sections the fibrin as seen in the umbilical cord takes an orange-red color, and the widely distributed fibrinoid material, which is so prominent in between the chorionic villi, is similarly stained. We could not determine any transition or continuity between either of these substances and the spirochete-like objects. In connection with the possibility of these structures resulting from the coagulation

of blood, the experimental studies of Stübel,<sup>14, 15</sup> and Barratt,<sup>16</sup> using darkfield illumination, have brought out the fact that in the course of coagulation of plasma the experimental conditions as well as the species of animal supplying the blood determine the character of the structures formed, *i.e.*, the fibers of fibrin may be coarse or fine, or fibrillar structures may not appear at all. Furthermore, Nageotte<sup>17, 18</sup> demonstrated that fibrin is not normally impregnable with silver, but if citrated plasma is mixed with calcium chloride, the fibrils formed in the coagulum can be impregnated with silver. However, the fibrils studied by Nageotte are not constant in size and conformation, as are the structures under discussion.

That the observed argentophilic spirals are not cell boundaries is established by the fact that the so-called "cell-boundaries" are not impregnated with silver after fixation by formalin.

Elastic fibers, other than those occurring in the walls of blood vessels, are not evident in the placental substance. They certainly are not found in the "spirochetal" foci, and the spirochete-like objects have not been found in the walls of the blood vessels, *i.e.*, in the places where the elastic fibers are known to occur. Moreover, elastic fibers are not argentophilic.

The above observations were fully supported by study of the control syphilitic tissue.

With the above possible sources of confusion ruled out, we conclude that in so far as the purely morphological evidence can be interpreted, the observed argentophilic spiralled structures are spirochetes. They agree in shape, size and spacing of coils with the morphological characteristics of the genus *Treponema*,<sup>19</sup> closely resembling two of its species, *T. pallidum* and *T. calligyrum*, which are known to occur in the genital tract of man. It is not possible from the present study to identify the spirochetes observed, but the striking fact, as mentioned above, is that they are found in cases that present clinically a suspicious history, whether it be abortion, prematurity, stillbirth or fetal anomaly. If this fact be more than a mere coincidence, the spirochetes found would belong either to a species of acknowledged pathogenicity, or to a species that has been considered non-pathogenic but which might be responsible for the lesions described in this paper.

## SUMMARY AND CONCLUSION

1. Placentas selected by the clinician because of a suspicion of syphilis have been studied by means of sections stained with hematoxylin and eosin, Weigert's elastica stain, silver impregnation, silver impregnation counterstained with Mallory's aniline blue, and silver impregnation partially desilverized by means of potassium iodide. The methods used were selected in order to facilitate the establishment of the nature of certain spirochete-like structures previously observed. The methods used are described.

2. The characteristics of the foci in which the spirochete-like objects are found have been described.

3. The possibility of the spirochete-like objects being elastic or collagenous fibers, reticular fibrils, fibrin, fibrinoid material, or cell boundaries has been ruled out.

4. It is concluded, on the basis of the purely morphological evidence offered, that the observed structures are spirochetes.

NOTE: I am indebted to Dr. H. G. Dorman for obtaining the placentas and for general suggestions concerning the problem; to Dr. E. Mayer for suggestions and remarks on the silver technics; to Dr. E. W. Dennis for help on the morphology of the spirochetes and the arrangement of the article; and to Mr. H. Berberian, for assistance with the microphotographs.

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## DESCRIPTION OF PLATE

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### PLATE 76

- FIG. 1. Microphotograph of a section impregnated with silver and counter-stained with Mallory's aniline blue stain for collagenous fibers, showing (a) an artery with periarteritis and endarteritis with collagenous fibers partially occluding the lumen, and (b) the border which stains orange-red and shows spirochetes at a higher magnification.
- FIG. 2. A microphotograph from the same slide as Fig. 1 showing periarteritis and endarteritis in five arterioles in a neighboring field.
- FIG. 3. A microphotograph of a preparation stained by the same technic as Figs. 1 and 2, and showing (a) a focus surrounded with argentophilic granules and spirochetes, (b) a portion of a degenerating villus, (c) a partially occluded blood vessel, and (d) small islets of collagenous fibers.

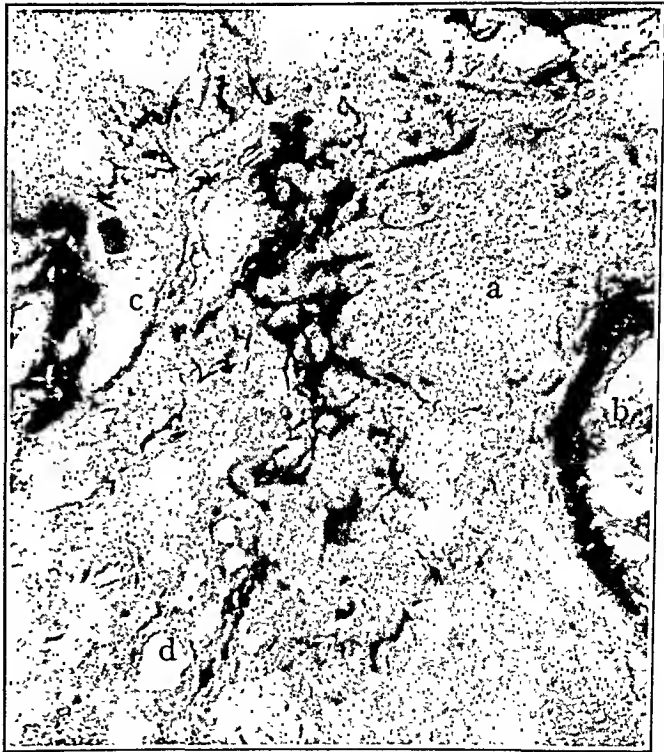


I



2

Sahyoun



3

Spirochete-Like Structures in Placenta



*Left Testis:* The epididymis was thickened and indurated and cross sections revealed numerous small abscesses.

*Brain:* The brain weighed 1440 gm. The contour was normal and the pia normal. Sectioning the brain showed an area of recent hemorrhage about 2 cm. in diameter located in the optic radiation of the left hemisphere of the forebrain. The hemorrhage was surrounded by a wide zone of edema with innumerable points of secondary capillary bleeding.

*Pituitary:* The pituitary was normal in size and contour. The basilar artery, the circle of Willis and the cerebral arteries presented normal walls. The contents were fluid blood.

*Anatomical Diagnoses:* (1) Chromaffin cell tumor (pheochromoblastoma) primary in the medullary portion of the right adrenal; neoplastic invasion of the lumen of the inferior vena cava; implantation metastases on the free surface of the overlying parietal peritoneum and on the contiguous peritoneal surface of the right leaf of the diaphragm; small implantation metastases on the free surfaces of both leaves of the mesentery of the small intestine, but no other distant metastases. (2) Cerebral hemorrhage, single, optic radiation of the left hemisphere of the forebrain. (3) Cystitis (urinary bladder), chronic, follicular; and (4) epididymitis, subacute, suppurative (left testis).

### *Comment*

The blood pressures in the above case are charted from 1924 to 1936 inclusive and are incorporated in Graph 2. The graph indicates that during 1930 there was a slight rise in both the systolic and the diastolic readings. The initial hospitalization of the patient was Nov. 15, 1929; the blood pressure of that date was 108/60 mm. Hg. From the onset of the final illness, Nov. 29, 1935, to death, which occurred Oct. 23, 1936, there were abrupt alterations in the blood pressure marked by daily and hourly changes, making the blood pressure graph resemble a temperature chart in a case of septicopyemia. The final illness was subacute in nature and the duration was approximately 11 months.

In explanation of the constipation and headache, two of the more prominent and distressing subjective manifestations, Wiggers,<sup>19</sup> in referring to the effect of epinephrin on muscle, writes that excitatory effects result on the pyloric and ileocolic sphincters.

Inhibition of contractions and tonus occurs in the gastrointestinal tract. In reference to headache, he states that increase in intraventricular pressure is believed to be the most common cause of headache. Intracranial and intraventricular pressure are simultaneously increased as a result of an increase in arterial pressure or reflex dilatation of the cerebral vessels. The headache so frequently associated with great hypertension becomes explicable on the grounds that increased arterial pressure *per se* increases brain volume and perhaps also increases the secretion of cerebrospinal fluid by the choroid plexus.

The episodes characterizing cases of paroxysmal hypertension associated with pheochromocytoma are, in my opinion, wholly explicable on the ground of the accumulation, in the substance of the newgrowth, of the secretory product (epinephrin) of the tumor cells. In any newgrowth there is not the normal physiological nerve control of the parenchyma and the normal mechanical removal of the secretory products by sinusoids is lacking; therefore the secretory products (if such be formed) will accumulate until large amounts gather and are "spilled over" into the capillary system of the neoplasia.

Almost all of the case reports illustrating paroxysmal hypertension (including that of the author) note some degree of tachycardia; some of the case reports indicate bradycardia. The pulse rate, in my opinion, indicates the amount of epinephrin "spilled over" during the episodes. (1) If the amount of epinephrin liberated be very great, sufficient to escape complete oxidation during its passage through the lungs, bradycardia will result. (2) If the amount of epinephrin liberated during the episodes be entirely or almost completely oxidized during its passage through the pulmonary circulation, tachycardia will result. Marey's law, quoted from Howell,<sup>20</sup> reads as follows: "Marey (La Circulation du Sang, Paris, 1881) gives the two following laws: (1) Whatever increases or diminishes the *force* with which blood is driven from the heart toward the periphery will cause the velocity of the blood and the pressure in the arteries to vary in the same sense. (2) Whatever increases or diminishes the *resistance* offered to the blood in passing from the arteries to the veins will cause the velocity and the arterial pressure to vary in an inverse sense. That is, an increased

resistance diminishes the velocity in the arteries while increasing the pressure, and *vice versa*." The slowing of the pulse after the intravenous administration of adrenalin is probably accounted for by secondary reflex vagus excitation because it does not occur (experimentally) after preliminary vagus section.

Starling<sup>21</sup> states: "The point of attack of adrenalin appears to be in the muscular or glandular tissue, since its effects may not only be obtained after destruction of the cord and sympathetic plexuses, but even obtained (and in an exaggerated degree) after time has been allowed for the peripheral (post-ganglionic) fibers to degenerate as a result of extirpation of the corresponding ganglia."

Referring to the transient glycosuria noted during or immediately after episodes of paroxysmal hypertension, I believe the glycosuria to be strictly renal in type, due to the sudden increase in pressure in the "arterial mirabile" of the kidneys (glomerular tufts of the renal corpuscles, including the afferent and efferent glomerular arterioles). Starling states that adrenalin acts directly on the liver cells, inciting a rapid discharge of glycogen with resulting hyperglycemia and glycosuria. In the 12 cases of paroxysmal hypertension cited, transient glycosuria was noted only in 3, and the highest blood sugar estimation was 125 mg. per cent, within the normal limits.

### SUMMARY AND CONCLUSIONS

The main object of the foregoing discussion of essential hypertension and paroxysmal hypertension, with reports of contrasting cases, is to assist in the differential diagnosis between two conditions which may be said to be similar yet entirely different from one another. The early recognition of the physiological response to pheochromocytoma primary in the adrenal medullary parenchyma is of great therapeutic importance by reason of the complete cure afforded by surgical intervention in a fairly high percentage of cases, contrasting with the poor prognosis offerable individuals with essential hypertension even of the benign type.

The following are the more apparent differential points dis-

tinguishing the two conditions (essential and paroxysmal hypertension) as exemplified by the case reports:

	ESSENTIAL HYPERTENSION	PAROXYSMAL HYPERTENSION
(a) Family history	Often strongly positive for chronic cardiovascular disease	Usually negative
(b) Previous illness	Previous hospitalization for cardiovascular disease may be listed	Usually irrelevant
(c) Present illness	Marked chronicity	Course relatively short and marked by episodes increasing in frequency and severity
(d) Special examinations		
X-Ray	Left ventricular cardiac hypertrophy. The aortic arch may be widened	Heart not necessarily enlarged; aorta may be normal
Electrocardiogram	Left axis deviation and T wave negativity may be noted	Not necessarily altered from normal except during episodes
Blood pressure	See Graph 1	See Graph 2
Pyelogram	Negative	Downward displacement of kidney on the side of the adrenal tumor mass may be noted
Blood counts	Slight secondary anemia	May be normal
Urine examinations	Traces of albumin with hyaline casts may be reported	Normal except during episodes when there may be transient renal glycosuria
Retinoscopy	Marked retinal angiosclerosis with hemorrhages	Retinal hemorrhages may be noted
(e) Causes of death	Cerebral accident; coronary accident; uremia; intercurrent disease. Death usually during the fifth or sixth decade of life	Cerebral accident which may be atypical in location. Congestive heart failure (left ventricular failure). Death during the fourth decade of life, at 34 years average of cases listed

Other conditions which must be ruled out in arriving at the recognition of pheochromocytoma cell tumor primary in the adrenal medullary parenchyma include: (1) Hypertension associated with renal arteriosclerosis, the so-called malignant phase of essential hypertension; (2) renal hypertension associated with the secondary contracted kidneys seen in chronic glomerular nephritis; (3) congenital and acquired forms of renal maldevelopment, in-



cluding polycystic kidneys; (4) neoplastic hyperplasia of the adrenal cortical parenchyma (hypernephroma and Grawitz tumors); and (5) brain tumors.

The reported case of paroxysmal hypertension was not diagnosed ante mortem, having been overlooked by competent observers; however, the consolation remains that the pheochromoblastoma found at autopsy was quite inoperable, due to the fact that (see Figure 1) it had invaded the lumen of the inferior vena cava and was not entirely benign in character, innumerable small implantation metastases already having made their appearance.

In the foregoing article the subject of essential hypertension has hardly been touched upon and it is my intention, in a series of subsequent articles, to report contrasting cases of essential hypertension of the so-called malignant type and nephritic hypertension, and to discuss and illustrate the more commonly encountered syphilitic cardiovascular reactions found at autopsy.

NOTE: I wish to express my appreciation to the Curator of the Army Medical Museum, Washington, D. C., for his assistance in the necessary microphotography.

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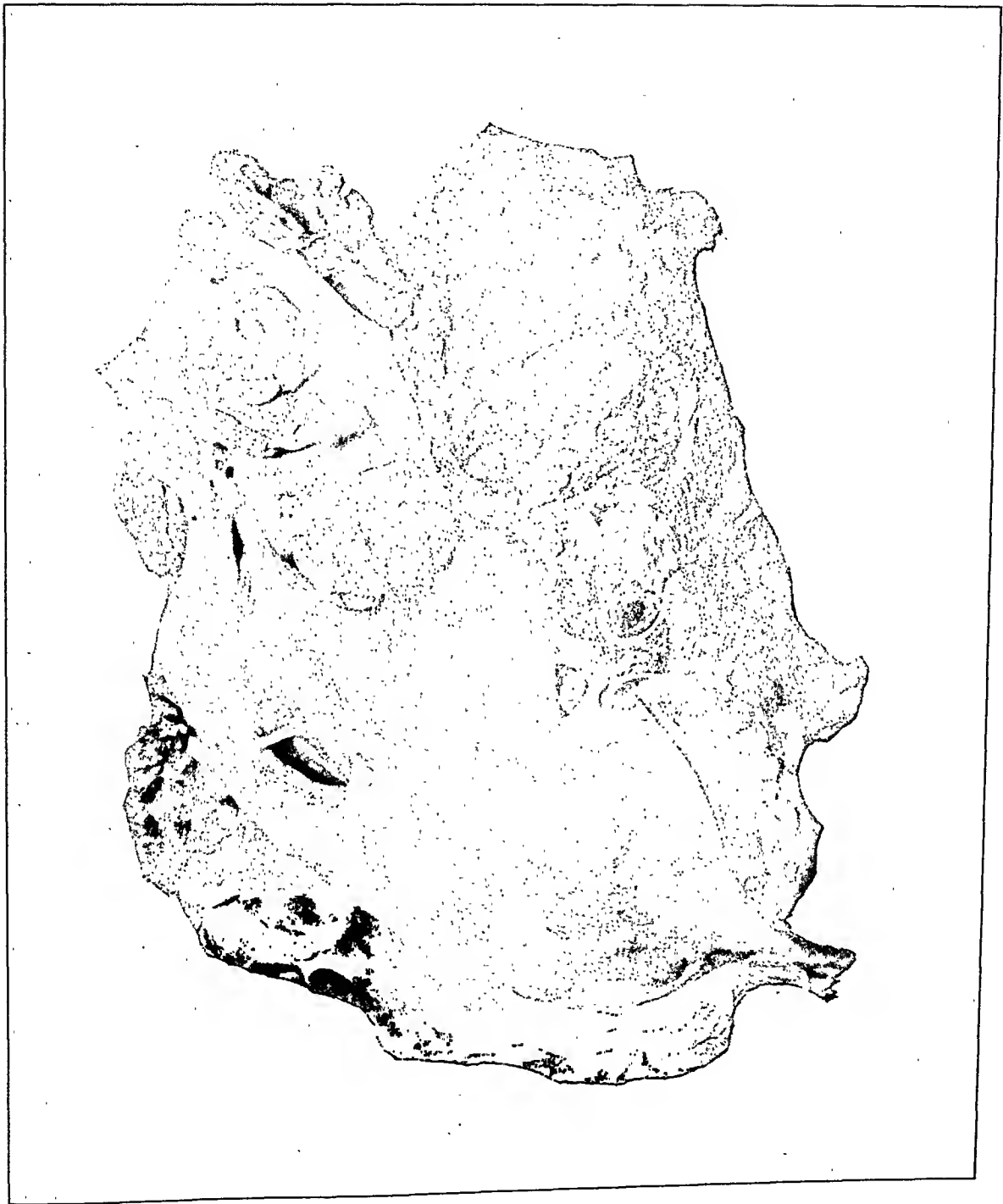
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### DESCRIPTION OF PLATES

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#### PLATE 110

FIG. 1. Pheochromoblastoma primary in the medullary portion of the right adrenal (Case 2, A-2018 W.R.G.H.). Posterior aspect of the tumor mass. The inferior vena cava is opened longitudinally. The growth invades the lumen of the cava in the form of an ovoid nodule about 0.5 by 2 cm. The neoplastic tissue was firm in consistence, the cut surfaces brownish gray, changing to a darker brown after having been immersed in Kaiserling I.



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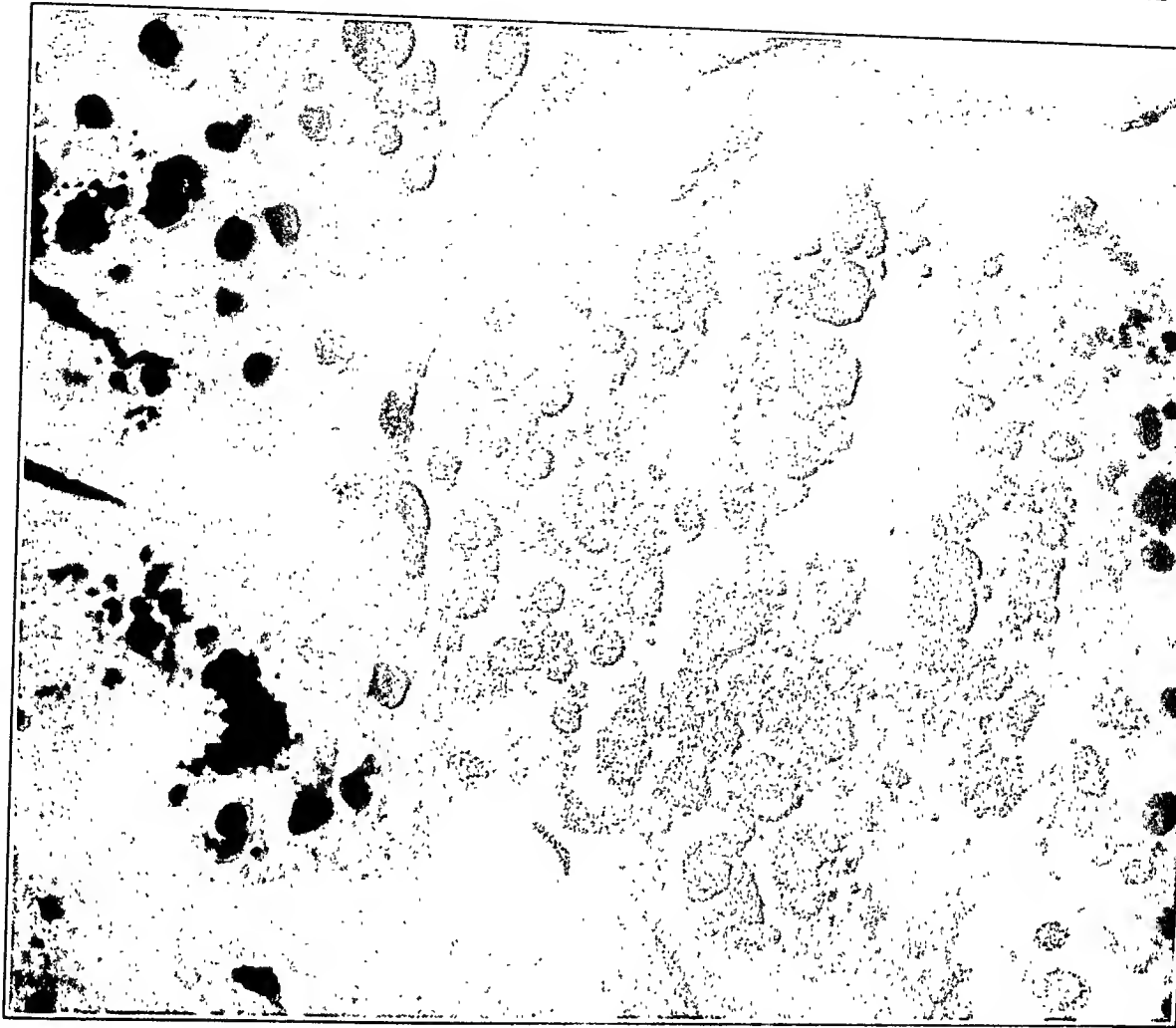
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Essential and Paroxysmal Hypertension

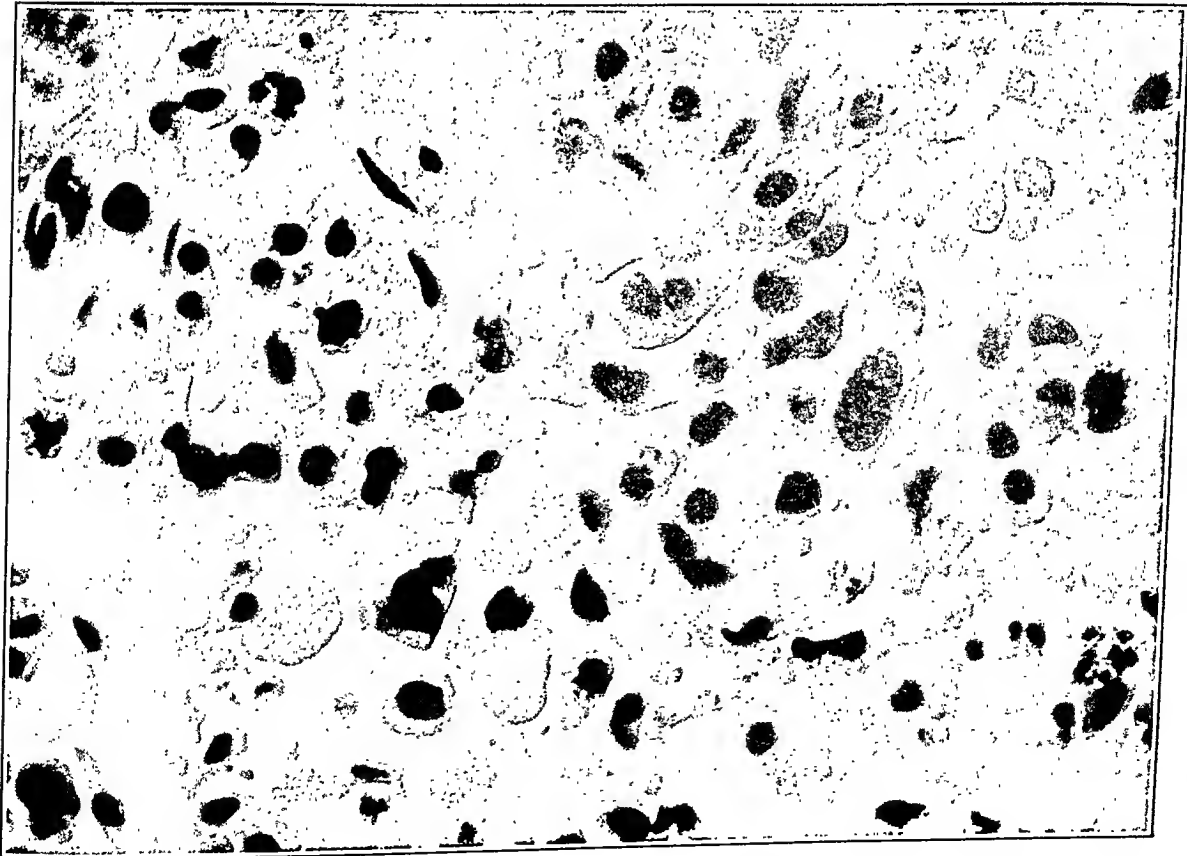
PLATE III

FIG. 2. Pheochromoblastoma primary in the medullary portion of the right adrenal (Case 2). Microphotograph showing one of the scattered collections of iron-free pigment regarded as melanin. Hematoxylin-eosin stain.  $\times 800$ .

FIG. 3. Pheochromoblastoma primary in the medullary portion of the right adrenal (Case 2). Microphotograph showing the irregularity in the size of the nuclei, the poorly differentiated nuclear detail, the tendency to eccentricity in nuclear placement, and the stippling of the cytoplasm of some of the cells. Hematoxylin-eosin stain.  $\times 810$ .



2



3



# IRON HEMATOXYLINS CONTAINING FERRIC AND FERROUS IRON\*

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Following our previous studies<sup>1</sup> on the superior keeping qualities of Janssens' iron hematoxylin and the demonstration of the preservation of the blue iron lake of hematoxylin by addition of a ferrous salt to the Janssens' formula, it seemed necessary to explore further the optimum concentrations and proportions of the various components of the mixture.

Experiment I was designed to compare the availability of ferrous sulphate in place of the ferrous ammonium sulphate used by Earle.<sup>2</sup>

TABLE I  
*Composition of Solutions in Experiment I*

Solution	I-1	I-2	I-3	I-4	I-5	I-6
(A) Ferric ammonium sulphate	1.0	2.5	10.0	1.0	2.5	10.0 gm.
Ferrous ammonium sulphate	1.0	2.5	10.0	..	..	.. gm.
Ferrous sulphate	..	..	..	1.0	2.5	10.0 gm.
Distilled water	100	100	100	100	100	100 cc.
(B) Hematoxylin	Solutions A and B were mixed on August 8, 1938.					
Alcohol, 95%						
Glycerine, C P						

Six solutions were used, the 1st containing 1 gm. each of ferric ammonium citrate and of ferrous ammonium citrate in 100 cc. of distilled water, and 1 gm. of hematoxylin in 50 cc. each of glycerine and of alcohol. In the 2nd solution the iron salts were increased to 2.5 gm. each, and in the 3rd to 10 gm. each. In the 4th, 5th and 6th solutions ferrous sulphate was substituted for ferrous ammonium sulphate in 1, 2.5 and 10 gm. quantities respectively. These solutions were made up on August 8, 1938.

All solutions remained blue-violet for at least 24 hours, but by August 10 the 4 weaker solutions had turned brown. Staining with the freshly prepared solutions gave sharp nuclear staining in 5

\* Received for publication August 4, 1939.

minutes with all solutions, but with Solutions 3 and 6, those containing the most iron, there was less staining of other tissue elements. Staining with Solutions 3 and 6 was still excellent after 8 days and 40 days. In about 6 weeks Solution 3 had lost its blue-violet color, becoming purple, and after 3 months, purplish brown. At this time Solution 6 was still violet. Work was interrupted from this time until July, 1939, the solutions remaining closed with cork stoppers on a window sill over a steam radiator. In July, 1939, all solutions were brown, but Solution 6 still gave fair nuclear staining.

It appeared that ferrous sulphate could be substituted for ferrous ammonium sulphate and that fairly high salt concentrations were necessary for stability of the solutions. The superiority of the simple ferrous sulphate solution is not improbably due to the higher iron content of the 10 gm. of the salts used (2.01 versus 1.42 gm.). The ferric iron content was 1.16 gm.

Experiment II was designed to test the proper proportion of the hematoxylin to the total iron salts. A solution of ferric ammonium sulphate, 17.27 gm., and ferrous sulphate, 9.96 gm. per 100 cc. of water, was made up. This gives 2 gm. each of ferric and ferrous iron as Fe. This iron solution was mixed with varying amounts of distilled water to make 20 cc. quantities, and 20 cc. of 1 per cent hematoxylin in glycerine and alcohol was added on August 9, 1938.

Solution 1 gave satisfactory staining in 5 minutes, Solutions 2 to 9 overstained in 5 minutes, Solutions 10 to 14 gave fully satisfactory staining in 5 minutes, and in 30 minutes Solutions 10 and 11 gave slight overstaining, and Solutions 12, 13 and 14 fully satisfactory staining.

All solutions were blue-violet when made up; in 1 day the first 3 were brown, in 1 week 2 more, in 5 weeks the first 7 were brown and the last 4 violet. In 3 months the last 2 were purple, the rest purplish to yellowish brown, and by July, 1939, all were brown with a little pinkish tinge in 13 and 14. Staining on July 26, 1939 showed Solutions 13 and 14 fully satisfactory, 12 good, and 9, 10 and 11 fair.

This test indicated that relatively high concentrations of the iron salts in relation to the amount of hematoxylin are necessary for stability.

Adopting a level of 1.75 gm. of ferric iron as adequate from



TABLE II  
Schedule of Dilutions and Iron Content of Solutions in Experiment II

Solution	II-1	II-2	II-3	II-4	II-5	II-6	II-7	II-8	II-9	II-10	II-11	II-12	II-13	II-14
Ferric ferrous iron solution	1	2	3	4	5	6	7	8	9	10	12.5	15	17.5	20
Distilled water	19	18	17	16	15	14	13	12	11	10	7.5	5	2.5	0
Gm. of Fe <sup>++</sup> and of Fe <sup>+++</sup> per 100 cc.	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.25	1.5	1.75	2.0

Experiment II, Experiment III was designed to test the proper amount of ferrous iron.

The following solutions were used to make each test solution: (1) A solution of 15.12 gm. of ferric ammonium sulphate in 52 cc. of distilled water. (2) A solution of 19.92 gm. of ferrous sulphate in 48 cc. of distilled water, or a part of this solution and enough additional water to make 48 cc. (3) A solution of 1 gm. of hematoxylin in 50 cc. each of glycerine and alcohol. Each solution of a final volume of 200 cc. contained 1.75 gm. of ferric iron, 1 gm. of hematoxylin and a quantity of ferrous iron varying from none to 4 gm., so that Solution A contained 0.1 gm., B, 0.2, and so on to J, 1 gm., K, 1.2 gm., and so on to O, 2 gm., P, 2.5, Q, 3, R, 3.5 and S, 4 gm. T lacked ferrous iron. Mixtures were made on August 10, 1938, and all were blue-violet when made. In 5 hours T was brown, A and B, purplish brown. By the next day all solutions with 0.8 gm. or less of ferrous iron were brown, those with 0.9 and 1, purplish brown, and the rest violet or blue-violet. In a week solutions with 1.8 gm. or more ferrous iron were still violet or blue-violet, in a month 2 gm. was the lower limit of violet, with little further change in 3 months. In July, 1939, solutions with 3, 3.5 and 4 gm. of ferrous iron were still distinctly purplish.

On the 7th day solutions with 1.8 gm. and more of ferrous iron gave optimal staining either in 5 or in 30 minutes. The control with no ferrous iron was distinctly poor, better in 30 minutes than in 5, and solutions with about 0.5 to 1 gm. ferrous iron were good in 5 minutes, optimal in 30. After 11½ months the last 3 solutions still gave excellent staining either in 5 or in 30 minutes, Solution P with 2.5 gm. of ferrous iron being distinctly inferior.

When it had appeared that in order to give a stable iron hematoxylin which did not overstain, a solution containing 15 gm. ferric ammonium sulphate (1.74 gm. ferric iron) and 15 gm. ferrous sulphate (3.01 gm. ferrous iron) in 100 cc. of distilled water, and 1 gm. of hematoxylin in 50 cc. of alcohol and 50 cc. of glycerine would be satisfactory, Experiment IV was set up to determine whether this solution could be diluted with water, or water, alcohol and glycerine without impairing its stability. The water, alcohol and glycerine diluent was made in the proportion 50:25:25. 5:5, 4:6, 2:8 and 1:9 dilutions were made with each diluent on August 24, 1938.

All dilutions deteriorated more rapidly than the stock solution, pure aqueous dilutions faster than the water, alcohol and glycerine dilutions and the higher dilutions fastest. While the stock solution had shown but little more intense staining in 30 than in 5 minutes, the aqueous dilutions showed marked overstaining when staining was prolonged. The water, alcohol and glycerine dilutions showed a less measure of the same change.

Since satisfactory myelin staining of previously chromated brain tissue may be obtained by overstaining with a neutral iron chloride or an iron alum hematoxylin, followed by differentiation with iron alum until gray and white substance can be distinguished, then treating with borax ferricyanide to develop the blue-black color of the myelin, it was thought that the overstaining with iron hematoxylin and the iron alum differentiation might be replaced by staining with an iron hematoxylin of the type under discussion which does not overstain. Solution III Q was used. This contains 15.12 gm. of ferric ammonium sulphate (1.75 gm. of ferric iron), 14.94 gm. of ferrous sulphate (3 gm. ferrous iron), 100 cc. of water; and 1 gm. of hematoxylin, 50 cc. of alcohol and 50 cc. of glycerine. Brain sections were stained 5, 10, 15 and 30 minutes, then treated 5 minutes with Weigert's borax ferricyanide. The staining effect was that of a plain iron hematoxylin stain, myelin not being colored at all.

#### DISCUSSION AND SUMMARY

The foregoing experiments show that iron alum hematoxylin solutions containing 13 to 17.3 gm. of ferric ammonium sulphate (1.5 to 2 gm. ferric iron) in a 200 cc. quantity stain intensely in 5 minutes and do not overstain appreciably in 30 minutes. The addition of 15 to 20 gm. of ferrous sulphate (3 to 4 gm. ferrous iron) preserves the original blue-violet color of the fresh solution for at least 3 months and preserves satisfactory staining for at least 11½ months. Ferrous ammonium sulphate and ferrous sulphate may be used as sources of ferrous iron. Ferrous sulphate is to be preferred as it gives higher iron concentration for the same weight of salt and is more soluble.

It may be generally observed that solutions retaining their blue-violet color give satisfactory staining. Those showing purplish violet to purplish brown may also be quite usable, but those show-

ing a yellowish brown color in thin layers are generally inert.

Solutions containing 1.5 to 2 gm. of ferric iron in the 200 cc. unit quantity to 1 gm. of hematoxylin give adequate nuclear staining in 5 to 7 minutes and do not stain other tissue elements appreciably in 30 minutes. Solutions containing around 0.5 gm. of ferric iron (4.3 gm. ferric ammonium sulphate) stain promptly and intensely in 4 to 5 minutes and overstain in longer periods.

For preservation of the blue iron hematoxylin lake about twice as much ferrous iron as ferric is necessary, that is, quantities of ferrous sulphate or ferrous ammonium sulphate to give 3 to 4 gm. of ferrous iron (15 to 20 gm. ferrous sulphate).

A satisfactory solution which can be kept unchanged for several months is the following:

(A) Ferric ammonium sulphate (violet crystals)	15 gm.
Ferrous sulphate .....	15 gm.
Distilled water .....	100 cc.
(B) Hematoxylin .....	1 gm.
Alcohol, 95% .....	50 cc.
Glycerine, C P .....	50 cc.

Mix A and B in equal quantities.

While the foregoing type of solution is remarkably stable and effective, it is felt that greater stability can be attained as further studies now in progress indicate that such solutions may be attained.

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## THE EFFECT OF YEAST ON THE INCIDENCE OF CIRRHOSIS PRODUCED BY LEAD ARSENATE \*

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In a previous publication <sup>1</sup> were recorded the results of experiments in which copper arsenate, lead arsenate and sodium arsenate were employed to produce cirrhosis of the liver. It was found that these substances were effective as cirrhogenic agents for rabbits; furthermore, the incidence of cirrhosis could be reduced by certain diets.

When a diet of hay (alfalfa) and oats was fed the arsenates produced areas of necrosis in various parts of the liver lobules. These necrotic areas were sharply defined, the outlines of the necrotic cells were distinct and the cytoplasm did not stain with any of the dyes used. The necrotic cells did not contain any fat. With the doses of the arsenates employed the necrotic areas were situated most frequently adjacent to the portal region, often surrounded it, and in many instances extended from one portal region to another. Phagocytes collected about these necrotic liver cells. As the damaged liver cells disappeared, connective tissue was laid down and cirrhosis resulted.

When the diet of hay and oats was supplemented by the addition of carrots on one day and cabbage the next the incidence of cirrhosis was considerably reduced. With a diet of peeled white potatoes and white bread the incidence of cirrhosis was markedly reduced, even though the average amount of arsenic contained in the livers of these rabbits was greater than in those of the other two series.

It was then decided to determine whether other substances, added to the diet, might decrease the effect of arsenates on the liver. In the following experiments are reported the results of the addition of powdered brewer's yeast to the diet of rabbits given lead arsenate. This arsenate was chosen because it was so effective in producing cirrhosis and also because it is so widely used as an

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insecticide. The lead arsenate used was one of the commercial preparations for spraying and dusting and was from the same container as that used in the previous experiments. This had been analyzed and found to contain 98 per cent lead arsenate.

The experiment was carried out after the manner of those previously described. Only chinchilla rabbits were used; each weighed 1250 to 1500 gm. at the beginning of the experiment. Under ether anesthesia a piece of liver was removed to serve as a control for any alterations that might subsequently occur. The animals were allowed to recuperate for at least 2 weeks. They were given a diet of hay (alfalfa) and oats throughout the entire period of the experiment; this diet will hereafter be referred to as the standard diet. The arsenate was thoroughly mixed with some starch and was sprinkled on a small quantity of oats; after these had been eaten the standard diet was allowed in liberal quantity. The brewer's yeast was administered in the same manner as the arsenate and when given with the arsenate it was mixed with the same oats.

The biopsy specimens of liver were fixed in a solution of formaldehyde U.S.P. (1:10). The autopsy material was fixed in a formaldehyde solution (1:10), and in Zenker's solution without the addition of either acetic acid or formalin. Sections were stained with hematoxylin and eosin and with the stain for the differentiation of hemosiderin and hemofuscin (Mallory, Parker and Nye<sup>2</sup>). Frozen sections of the liver were stained with scharlach R. In addition, specimens of many of the livers were fixed in absolute and in 95 per cent alcohol, and sections from them were stained for glycogen according to Best's method. Arsenic determinations were made by the Gutzeit method. The yeast was analyzed for arsenic but only a trace was found.

#### STANDARD DIET

Four rabbits were fed the standard diet without the addition of lead arsenate or yeast. They were kept on the diet for 265 days, when they were sacrificed. The rabbits increased in size steadily and were very fat. The livers from this group of rabbits were free from cirrhosis. In 1 rabbit the pigment in the liver cells was slightly increased, in another the amount of pigment was not changed, and in 2 it was slightly decreased. There were no phago-

cytes in any of the livers. Arsenic was not present in any of the livers or kidneys (Table II).

#### STANDARD DIET AND YEAST

There were 5 rabbits in this series. Each was given 3 gm. of powdered yeast daily. The animals were sacrificed at the end of 384 days. At this time they were very large and fat, their fur unusually thick and long.

There were no hepatic lesions in this group nor were there any phagocytes. In 2 of the rabbits the hepatic pigment was not increased, in 2 it was slightly increased, while in the other there was a moderate increase of pigment. There was no arsenic in the livers or kidneys (Table II).

#### STANDARD DIET AND LEAD ARSENATE

Five rabbits were given daily doses of 7.2 mg. of lead arsenate (1.4 mg. of arsenic). The 1st animal of this group died on the 8th day and none lived longer than 26 days. In no instance could any gross abnormality of the liver be detected. In 2 of the rabbits (Nos. 410 and 412) a few areas of hepatic necrosis were found; in the other 3 animals areas of necrosis were numerous. These areas were, most often, adjacent to the portal regions, though they were found also in other parts of the lobule. Frequently the areas of necrosis extended from one portal region to another. They were sharply defined from the surrounding liver cells. The necrotic hepatic cells had distinct outlines, were swollen, somewhat rigid in appearance and the cytoplasm rarely stained with eosin. No nuclei could be seen in many of the necrotic cells; in other cells the nuclei were fragmented or pyknotic. These necrotic liver cells did not contain fat. The endothelium of the sinusoids in the areas of necrosis appeared unharmed. Large phagocytes were frequently collected about the necrotic liver cells. In some instances these phagocytes were numerous and many of them were multinucleated. At times similar phagocytes were present in the portal areas. There was as yet little evidence of the laying down of connective tissue in the areas of necrosis.

In the 2 rabbits (Nos. 412 and 417) that survived for 26 days the connective tissue of the portal areas was increased, but not strikingly so, and did not pass to any of the neighboring portal

areas. This is designated as early cirrhosis. The bile ducts were beginning to proliferate.

The pigment in the liver cells was not increased in 2 of the animals; 1 of these died on the 8th day, the other on the 15th day. There was a slight increase of pigment in the liver cells of the animal that died on the 14th day; in the other 2 rabbits the increase of the hepatic pigment was moderate. The phagocytes were often filled with pigment similar to that seen in the liver cells.

Another series of 7 rabbits was fed the standard diet and given lead arsenate. The daily dose of the arsenate for the first 52 days was 2.4 mg. (arsenic 0.4 mg.); the amount of the arsenate was then increased to 4.8 mg. (1 mg. arsenic). Two rabbits died after having received the increased doses of arsenate for 40 days; these animals had thus received arsenate over a period of 92 days. The other 5 rabbits were continued on the arsenate dosage of 4.8 mg. for 118 days. The amount of arsenate was then increased to 7.2 mg. (1.4 mg. arsenic); after 8 days on this dosage the animals were sacrificed. These 5 animals received the arsenate for 178 days.

Only 1 rabbit (No. 422) had a grossly normal appearing liver; of the other 6, the livers of 4 had an irregular capsular surface and on section the cut surface was browner than normal and appeared moist. The livers of the 2 remaining rabbits, while having a smooth external surface, had a moist edematous cut surface, and in 1 of these the lobulation was irregular.

In none of these 7 rabbits were areas of hepatic necrosis found nor were any individual liver cells necrotic. However, in 6 rabbits connective tissue in the portal areas was increased. In 4 animals the connective tissue did not pass to any of the adjacent portal areas; in 2, bands of connective tissue extended to neighboring portal areas. There was no irregularity of lobular outline in any of these livers. Following the classification used in the previous report to designate the degree of cirrhosis, the 4 examples in which the connective tissue was limited to the portal area are classified as early, the other 2 as moderate. The 1 rabbit in the group that had a liver free from cirrhosis was No. 422.

Phagocytes were found in each of the livers, although they were not so numerous as in the other rabbits with areas of hepatic



necrosis. The phagocytes were present in the portal areas and sinusoids; many of them were multinucleated.

Hepatic pigment was not increased in the liver of 1 rabbit (No. 425); in the others the pigment was increased, although not markedly so. It was present in hepatic cells, Kupffer cells and phagocytes. There was proliferation of bile ducts in 4 of the rabbits (Nos. 416, 423, 425 and 426). The quantities of arsenic found in the livers and kidneys are shown in Table I.

#### STANDARD DIET, LEAD ARSENATE AND YEAST

Thirteen rabbits were fed the standard diet and to each of them was given daily 7.2 mg. of lead arsenate (1.4 mg. of arsenic) and 3 gm. of yeast. In Table I are shown the various intervals the rabbits survived. It will be noted the first death occurred on the 58th day. Rabbits Nos. 374 and 379 were sacrificed on the 384th day to terminate the experiment.

The external surface of the liver of each of these rabbits was normal; in only 3 instances (rabbits Nos. 370, 371 and 407) could any increase of connective tissue in the portal areas be detected when the liver was sectioned. Necrotic areas such as have been described in the animals given similar daily doses of the arsenate were found in only 3 of the livers. These areas were very few in number and were situated in various parts of the lobule. Necrosis of individual liver cells was found only in the liver of 1 rabbit (No. 374). These necrotic cells were situated near the portal regions and their appearance was similar to that of the cells in areas of necrosis. The connective tissue of the portal area was somewhat increased in 3 of the livers but did not, however, pass to any of the adjacent lobules and the cirrhosis is therefore designated as early. In 3 other instances there was a greater increase of connective tissue in the portal areas with strands of connective tissue at times passing to nearby portal areas (moderate cirrhosis). Proliferation of bile ducts was found in 5 instances (rabbits Nos. 370, 371, 375, 380 and 407), but the proliferation of the ducts was not a striking feature. It will be noted that this duct proliferation occurred only in those livers that were cirrhotic.

Phagocytes were not found in 4 of the livers (rabbits Nos. 366, 372, 375 and 378); a few were found in 3 (Nos. 369, 373 and 379), and they were more numerous in the other livers, especially

those in which cirrhosis was present. The phagocytes were situated near the necrotic areas, in portal areas and in the sinusoids, and were at times multinucleated.

Pigment was increased in the liver cells of 11 of the animals;

TABLE I

*Résumé of Hepatic Lesions in Rabbits Given Lead Arsenate or Lead Arsenate and Yeast*

Rabbit No.	Lead arsenate, daily dose	Days	Necrotic areas in liver	Cirrhosis	Arsenic	
					Liver	Kidney
<i>Lead Arsenate</i>						
	<i>mg.</i>				<i>mg. per 100 gm. dry wt.</i>	
413	7.2	8	Numerous	None	0.500	
409	7.2	14	"	"	0.500	0.320
410	7.2	15	Few	"	0.500	0.640
412	7.2	26	"	Early	2.093	1.230
417	7.2	26	Numerous	"	2.350	4.050
425	a	92	None	Moderate	0.667	0.400
426	a	92	"	"	0.667	0.400
415	b	178	"	Early	0.500	0.500
416	b	178	"	"	0.667	0.532
419	b	178	"	"	Traces	Traces
422	b	178	"	None	0.500	0.400
423	b	178	"	Early	0.500	0.250
<i>Lead Arsenate and Yeast</i>						
372	7.2	58	None	None	...	....
366	7.2	62	"	"	....	....
378	7.2	62	"	"	....	....
370	7.2	98	Few	Moderate	1.000	....
377	7.2	137	None	Early	0.170	....
371	7.2	154	Few	Moderate	1.700	1.320
407	7.2	172	None	"	1.000	0.440
369	7.2	174	"	None	1.330	0.600
373	7.2	178	"	"	1.000	0.908
380	7.2	181	Few	Early	0.660	0.276
375	7.2	310	None	"	0.165	0.200
374	7.2	384	"	None	0.330	0.332
379	7.2	384	"	"	0.267	0.224

a = 2.4 mg. lead arsenate (0.4 mg. arsenic) daily 52 days.

4.8 mg. lead arsenate (1.0 mg. arsenic) daily 40 days.

b = 2.4 mg. lead arsenate (0.4 mg. arsenic) daily 52 days.

4.8 mg. lead arsenate (1.0 mg. arsenic) daily 118 days.

7.2 mg. lead arsenate (1.4 mg. arsenic) daily 8 days.

it was also seen frequently in Kupffer cells and usually in the phagocytes.

The arsenic determinations of the livers and kidneys are shown in Table I.

## PIGMENT

Pigment was present in biopsy specimens of the liver from each of the rabbits. It was finely granular, yellow and refractive. The amounts and distribution varied in different animals. The pigment did not contain iron.

The pigment found in the livers at autopsy had the same appearance as that in the biopsy specimens although the granules were frequently larger. It did not give a reaction for iron but stained red with scharlach R. The greatest increases of pigment were in those animals that received the arsenate.

## GLYCOGEN DETERMINATIONS \*

Glycogen analyses were made on the livers of the 4 diet control rabbits, the 5 rabbits given the standard diet and yeast, 5 of the rabbits given lead arsenate, and 2 of those that received lead arsenate and yeast. The results are shown in Table II, together with the amounts of arsenic found in the livers and kidneys. The glycogen was determined by the method of Good, Kramer and Somogyi.<sup>3</sup> The animals were killed in the forenoon. Care was exercised to obtain the specimens of liver for glycogen analysis as quickly as possible after the animals had been killed. It will be noted from a study of the table that there is great variation in the amounts of glycogen found in different animals subjected to the same experimental procedure, as well as in those of different groups. The greatest average amount was found in the group of rabbits that received yeast; the next largest average amount was in the livers of the animals that received arsenate. The average amount of glycogen found in the livers of the arsenate-yeast rabbits was comparable to that of the diet control group. There was no correlation between the glycogen and arsenic content of the livers.

Sections of liver were stained by Best's method and the distribution of glycogen within the liver lobule was studied. Among the diet control group, with one exception, glycogen was found in the liver cells throughout the lobule, although it was most abundant in those cells surrounding the efferent veins. The one exception was rabbit No. 343; in this rabbit the glycogen could be demon-

\* We are greatly indebted to Dr. Louis B. Dotti for assistance with the glycogen determinations.

strated only in widely separated liver cells situated in various parts of the lobule.

The most regular distribution and even concentration of glyco-

TABLE II  
*Glycogen Content of the Liver*

Rabbit No.	Days	Glycogen content of liver	Arsenic		Cirrhosis
			Liver	Kidney	
Diet Controls					
		%	mg. per 100 gm. dry wt.		
343	265	1.000	o	o	None
339	"	1.750	o	o	"
341	"	1.845	o	o	"
354	"	2.135	o	o	"
		<u>1.682</u>			Average
Yeast Controls <sup>1</sup>					
405	384	1.860	o	o	"
406	"	2.510	o	o	"
401	"	2.810	o	o	"
404	"	3.260	o	o	"
402	"	3.540	o	o	"
		<u>2.796</u>			Average
Lead Arsenate <sup>2</sup>					
415	178	1.570	0.500	0.500	Early
423	"	1.710	0.500	0.250	"
422	"	2.040	0.500	0.400	None
416	"	2.330	0.667	0.532	Early
419	"	2.920	Traces	Traces	"
		<u>2.114</u>			Average
Lead Arsenate and Yeast <sup>3</sup>					
374	384	1.000	0.330	0.332	None
379	"	2.000	0.267	0.224	"
		<u>1.500</u>			Average

<sup>1</sup> 3 gm. powdered brewer's yeast daily.  
<sup>2</sup> 2.4 mg. lead arsenate (0.4 mg. arsenic) daily 52 days.  
 4.8 mg. lead arsenate (1.0 mg. arsenic) daily 118 days.  
 7.2 mg. lead arsenate (1.4 mg. arsenic) daily 8 days.  
<sup>3</sup> 7.2 mg. lead arsenate (1.4 mg. arsenic) daily 384 days.  
 3 gm. powdered brewer's yeast daily 384 days.

gen were found in the livers of the rabbits that received yeast. In this group of animals each liver cell in the lobule appeared to hold an equal amount of glycogen.

A definite and uniform irregularity of glycogen deposition was

found in 3 of the rabbits that received only the arsenate. About most of the portal areas in the sections of liver from each of these animals (rabbits Nos. 415, 422 and 423) was a zone where the liver cells had no demonstrable glycogen. These zones were several cells wide and frequently extended to the adjacent portal areas. These glycogen-free cells in the hematoxylin-eosin preparations had well preserved, normal appearing nuclei and were of the same size as the hepatic cells about the efferent veins. The only difference that could be detected was that the cytoplasm of the glycogen-free cells was less granular than that of the cells filled with glycogen; this difference, however, was not striking. The glycogen in the livers of the other 2 animals of this group was uniformly distributed throughout the lobule.

In 1 of the 2 rabbits (No. 374) that was given arsenate and yeast the glycogen was deposited chiefly in the liver cells about the efferent veins and portal areas; in the midzonal portion of the lobules glycogen was much less abundant and was in widely separated single cells. In this animal an occasional isolated cell, immediately adjacent to the portal areas, had sharply defined borders and clear cytoplasm. These cells resembled those of the necrotic area produced by arsenic in their staining with hematoxylin and eosin. In the section stained by Best's method similar cells were observed to be more uniformly filled with glycogen than were the neighboring liver cells. This suggests the possibility that the damaged hepatic cells in the necrotic areas are filled with glycogen. The glycogen in the liver of the other animal of this group was found in the hepatic cells throughout the lobule but the deposition was greatest in those cells about the efferent vein.

#### DISCUSSION

There are obvious differences in the groups of rabbits that received the arsenate and those given the arsenate and yeast. Of the 5 rabbits given 7.2 mg. of the arsenate daily, none lived longer than 26 days and of these 2 had early cirrhosis. The characteristic areas of necrosis were found in each of the livers, being very numerous in 3 rabbits. In the second control group given smaller amounts of the arsenate, necrosis was not found although cirrhosis was present in 6 of these 7 rabbits. It would seem, therefore, that the arsenate in small doses will produce a slowly developing

cirrhosis without the sharply defined areas of necrosis. Among the group of 13 rabbits given 7.2 mg. of the arsenate and 3 gm. of yeast daily, the 1st animal to succumb died on the 58th day. This is more than twice the length of time the last survivors of the corresponding control group lived. The addition of yeast had a distinct effect in prolonging the lives of the rabbits. Another difference noted is in the frequency of the characteristic areas of hepatic necrosis. These were found in only 3 of the 13 rabbits given yeast and arsenate. Furthermore, these areas were few in number, while in the 5 animals given a similar amount of the arsenate without yeast they were very numerous in 3.

When the incidence of cirrhosis in the group of 13 rabbits given the arsenate and yeast is compared with that in the group receiving the smaller doses of the arsenate, a difference is apparent. In the arsenate and yeast series 6 of the 13 animals had cirrhosis, an incidence of approximately 46 per cent; in the group given only the arsenate, cirrhosis was found in 6 of the 7 rabbits, approximately 85 per cent.

The amounts of glycogen in the livers is so variable no very definite conclusion can be drawn. The highest average value was found in the livers of the animals given yeast daily as a supplement to the standard diet. That the increased glycogen content of these livers may be attributed to the yeast is indicated from the experiments of Bickel and Nigmann,<sup>4</sup> Bickel and Collazo,<sup>5</sup> and Labbé, Nepveux and Gringoire.<sup>6</sup> But this explanation cannot be offered for the somewhat higher average of glycogen content in the liver found in the rabbits that received arsenate only, as compared with those of the diet controls.

The lower incidence of cirrhosis among the animals given the arsenate and yeast is in accord with results of the recently reported experiments of Nakahara, Fujiwara and Mori.<sup>7</sup> These investigators using dimethyl-amino-azo-benzol to produce liver cell carcinoma in rats found that the incidence of cirrhosis and carcinoma was reduced when the diet of polished rice was supplemented by yeast.

What the protective substance in the yeast may be has not yet been determined.

## CONCLUSIONS

The incidence of hepatic cirrhosis in rabbits produced by the ingestion of lead arsenate is reduced when powdered brewer's yeast is added to the diet.

There is no apparent relation between the amount of hepatic glycogen and the quantity of arsenic in the liver, nor is there any obvious connection between the glycogen content of the liver and the incidence of cirrhosis.

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# PRIMARY CHORIONEPITHELIOMA OF THE URINARY BLADDER IN A MALE \*

## REPORT OF A CASE

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Truly ectopic chorionepithelioma in the male is rare. Up to 1933 Heaney<sup>1</sup> found 131 reported cases of chorionepithelioma in the male and with a few exceptions the primary tumor was in the testicle. Only 6 cases of ectopic chorionepithelioma in which the testes were carefully examined and definitely excluded as the primary site have been reported to date. The following case makes the 7th reported to date.

## REPORT OF CASE

*Clinical History:* J. J., a white male, aged 70 years, formerly a hospital orderly, was admitted to the urological service of The Mount Sinai Hospital Dec. 1, 1938, with the complaint of hematuria, dysuria and frequency. Four months before admission he noticed discomfort on urination and a sensation of "gravel" in the urethra. Two months later he began to have frequency, urinating every 3 to 5 minutes, and noticed that the urine was blood tinged. At times blood clots would form and prevent urination, the latter being completed only after the passage of the clots, some of which measured 2.5 to 6.5 cm. in length. During this time there was severe pain on urination, which continued throughout micturition. Five weeks before admission to the hospital the patient felt as though the bladder were distended. On passing a catheter it was found that the presence of blood clots had prevented urination for almost 8 hours. A large amount of urine was obtained which was bloody and full of clots. For over a month the patient had also noticed occasional streaks of blood in the sputum. Five days before admission to the hospital marked dyspnea and cyanosis developed.

*Physical Examination:* This revealed a dyspneic and cyanotic elderly individual who manifested marked discomfort. No râles were heard in the chest. Examination of the heart was entirely negative. The blood pressure was 120 mm. Hg. systolic and 50 diastolic. The nipples were sensitive to the touch. There was general tenderness over the entire abdomen, particularly in the right upper quadrant, the epigastrium and the right lumbar region. The liver was easily palpable in the epigastric and right hypochondriac regions and was painful to the touch. The prostate was found to be moderately enlarged.

*Laboratory Data:* The hemoglobin (Sahli) was 50 per cent. The urine

\* Received for publication June 24, 1939.

† George Blumenthal, Jr. Fellow in Pathology.

was grossly tinged with blood, was alkaline in reaction and had a specific gravity of 1.020. The test for albumen was 1 plus. Culture of the urine revealed *Staphylococcus albus* A and enterococcus. A cystogram was done but was unsatisfactory because of an inadequate amount of opaque material within the bladder. The blood urea on admission was 28 mg. per cent. The venous pressure was 11 cm. with a rise to 14.5 cm. on pressure in the right upper quadrant of the abdomen. The saccharine circulation time was 17 seconds. An electrocardiogram revealed regular sinus rhythm with frequent auricular premature contractions. There was left ventricular preponderance.

*Course of Illness:* The temperature on admission was 101.4° F. Throughout the patient's stay in the hospital the temperature ranged between 101 and 102° F. Three days after admission it was noted that the patient showed evidence of heart failure on the right side. The veins of the neck were distended and the liver was enlarged to three finger breadths below the costal margin. There was pitting edema over the sacrum and legs. Bilateral basal râles were present in the chest. Digitalis and diuretics were prescribed. At this time an indwelling catheter was left in the bladder. One week after admission to the hospital the blood urea rose to 61 mg. per cent. A small transfusion was given but the patient's general condition became steadily worse. He passed into a delirium with Cheyne-Stokes respiration and died 12 days after admission. A cystoscopy to determine the cause of the hematuria was not attempted because of the precarious condition of the patient.

#### POSTMORTEM EXAMINATION

The autopsy was performed 1 hour and 15 minutes after death. The essential changes noted were as follows:

The sclerae showed a slight icteric tinge. The breasts were not enlarged and contained no palpable nodules. The distribution of hair was normal. A small dermoid cyst 1.5 cm. in diameter was present within the skin of the anterior abdominal wall in the midline in the epigastrium. The lower border of the liver was situated 10 cm. below the costal margin in the right midclavicular line. The bladder was of approximately average size and was rigid and firm on its posterior and right lateral aspects, being densely adherent to the right side of the pelvic wall. When the bladder was opened it was found to contain fragments of pinkish gray, friable material mixed with bloody fluid. A tumor mass measuring 7 by 8.5 cm. (Fig. 1) involved the right half of the trigone and the entire right and the greater portion of the posterior walls which were greatly thickened and rigid, varying in width from 2 cm. to approximately 5 cm. in the trigonal region. The mucosal aspect of the tumor presented a fungating, pinkish gray sloughing area which was raised above and distinctly demarcated from the more normal, smooth grayish pink mucosa. A

small, firm, grayish yellow ulcerated tumor nodule 3 mm. in diameter projected above the surrounding mucosa in the left fundal region. Section through the tumor mass presented a mottled, dark grayish red appearance. Other areas were distinctly hemorrhagic. When viewed from the cul-de-sac aspect an extensive, mottled, irregular hemorrhagic area was seen beneath the smooth and glistening peritoneum covering the posterior wall of the bladder. This area was moderately firm and slightly nodular to the touch. The right ureteral orifice could not be found nor could it be probed from above by introduction of a catheter into the ureter. The left ureteral orifice was patent but was slightly encroached upon by the hemorrhagic tumor mass which terminated in juxtaposition to it. The prostate was not enlarged and not involved by the tumor. The seminal vesicles were surrounded by tumor tissue. A small bilateral hydrocele was present. The testes were similar and normal in size and shape. Serial sections 1 to 2 mm. in thickness through both testes and epididymes failed to reveal any abnormal features. The spermatic and lower pelvic veins contained phleboliths. Both ureters were dilated, the right being larger and containing about 50 cc. of bloody, dark red urine. The right kidney was smaller than the left and showed a moderate degree of hydronephrosis with atrophy of the parenchyma. Both kidneys contained deeply hemorrhagic tumor nodules of varying size. A bilateral adrenonephric symphysis was present. The liver was markedly enlarged and weighed 3220 gm. Its surface was studded with numerous firm tumor nodules varying from 1 to 6 cm. in diameter. Some of these nodules had umbilicated centers, others were hemorrhagic in appearance, and a few were yellowish white with dark red flecks. Cut surface of the liver revealed replacement of a greater portion of the liver parenchyma by well demarcated tumor masses, most of which had a mottled, dark red honeycombed appearance. Most of the smaller nodules, however, were a pale yellow color with dark red streaks coursing through them. The lungs were voluminous and weighed 2860 gm. Rounded, nodular, bulging tumor masses varying in diameter from 0.5 to 6 cm. were seen on all surfaces of the lungs. A few nodules were subpleural, had umbilicated centers, and were a mottled dark red color. Deeply embedded masses were also present which produced distinct bulges on the surface of the lungs. These

tumor masses were covered by normal intervening parenchyma. On cut section (Fig. 2) each lung appeared riddled with distinctly demarcated tumor nodules of varying size which projected above the cut surface. The larger tumor nodules were friable and dark red, and appeared indistinctly honeycombed. The centers of the nodules were soft in consistence. The smaller tumor nodules were for the most part a pale yellow color with reddish areas. Between the nodules were areas of bronchopneumonia. Considerable emphysema was present. In the gross, similar metastatic tumor nodules were seen in the ribs, sternum and vertebrae, epicardium of the heart, spleen, adrenals, pancreas, omentum, mesentery, small intestine and the tracheobronchial, para-aortic and cervical lymph nodes. The pituitary was normal in size and on sagittal section revealed small flecks of soft whitish tissue about 1 mm. in diameter which appeared to be located at the junction of the anterior and posterior lobes. A single small hemorrhagic tumor nodule was found in the right occipital lobe of the brain.

### BIOLOGICAL EXAMINATION

*Urine:* The *rat test* for gonadotropic hormone gave a positive reaction with 0.1 cc. of urine, which is equivalent to 10,000 rat units per liter. This corresponds to the pregnancy titer.

The *rabbit test* disclosed more than 1000 rabbit units of gonadotropic hormone in each liter of urine, which is a strong reaction, as in the test for pregnancy 20 cc. of urine are required for a positive reaction. Tests for estrogenic hormone were negative.

*Tumor Tissue (Lung Metastases):* Extracts of tumor tissue from the lungs failed to show either gonadotropic or estrogenic activity.

### MICROSCOPIC EXAMINATION

Sections through the bladder in the region of the tumor mass showed the marked thickening to be due to infiltrating sheets of tumor cells which penetrated all coats of the bladder and extended posteriorly to surround the seminal vesicles. The superficial layers were necrotic and hemorrhagic. Similar areas were present in the deeper layers. The more intact portions were composed of cells resembling the Langhans' cells of normal placental tissue (Fig. 3). They were large polyhedral cells with a basophilic

vacuolated cytoplasm and large vesicular nuclei. Occasional mitoses were present. Scattered among the Langhans' cells were large cells with irregular outlines and an acidophilic cytoplasm containing multiple hyperchromatic nuclei which conformed in appearance with that of the syncytial cells of the placenta (Fig. 4). No cilia were found on their borders. Small nests of Langhans' cells were seen within submucosal blood vessels of the bladder and in vessels about the seminal vesicles. The nodules of tumor tissue in the other organs were similar histologically with the degree of necrosis and hemorrhage varying in the individual nodules. Those in the lungs and liver compressed the surrounding normal parenchyma. Nowhere were vessels seen within the tumor masses, the blood being free in spaces between sheets of tumor cells. Moderate amounts of glycogen were present within the Langhans' cells. Microscopic metastases were present in the thyroid and the posterior lobe of the hypophysis. The latter organ contained scattered groups of large, irregularly shaped cells suggestive of "pregnancy cells." The testes were examined by serial block section and showed no microscopic evidence of chorionepithelioma. Spermatogenesis was moderately active but scattered atrophic and fibrotic tubules were also seen. Nodular areas of interstitial cell hyperplasia were present. The parathyroids were normal. Areas of chronic and acute pneumonitis were present in the lungs.

### DISCUSSION

Reports in the literature of proved primary ectopic chorionepithelioma in the male in which testicular examinations have been thoroughly and completely carried out are few in number. Kantrowitz,<sup>2</sup> in addition to reporting an undoubted case primary in a teratoma of the mediastinum, reviewed the literature up to 1934 and found only 4 cases which fulfilled the criteria proposed by Prym<sup>3</sup> (thorough examination of the genital tract, particularly the testes) — those of Miller and Browne,<sup>4</sup> Krassnianskaya,<sup>5</sup> Arendt,<sup>6</sup> and Heaney.<sup>1</sup> Gerber<sup>7</sup> added 1 more case, making a total of 6 acceptable ectopic cases in the male. All tumors were situated either in the mediastinum, the lung, or retroperitoneally. Three were composed purely of chorionepitheliomatous elements, while the remaining three had a demonstrable origin in a teratoma.

Search of the literature for primary chorionepithelioma of the

bladder yielded 5 cases. Two were in males and 3 in females. Critical analysis of these cases makes it doubtful if all can be accepted as primary tumors of the bladder, or even as chorion-epitheliomas.

Djewitzki's <sup>8</sup> case was in a 75 year old virgin female, who had had vaginal bleeding for 2 years. Dysuria with reddish urine had also been present. Dilatation and curettage of the uterus revealed a hyperplastic endometrium. At autopsy multiple fibromyomas were found in the uterus. The ovaries were small and atrophic. The tubes and vagina were negative. Situated on the posterior wall of the bladder was an elevated, dark red, centrally ulcerated tumor measuring 5 cm. in diameter. Metastases were present in the lungs, bronchial lymph nodes, spleen and sigmoid. Microscopically both the tumor of the bladder and the metastases showed the typical appearance of Marchand's <sup>9</sup> chorionepithelioma. The examination seems to have been quite thorough and this must be accepted as the 1st reported case of chorionepithelioma of the bladder.

Venulet <sup>10</sup> reported the case of a male, aged 30 years, in whom autopsy revealed a polypoid papillary tumor 0.5 cm. in diameter at the right ureteral orifice. Metastases were present in the lungs, mesentery, retroperitoneal lymph nodes and the pelvic space posterior to the bladder. Ascites and hydrothorax were present. The testes were said to be negative but the thoroughness of their examination cannot be ascertained. Microscopically the tumor showed both Langhans' and syncytial cell groups, but the author did not believe he was dealing with a malignant chorionepithelioma because of lack of hemorrhage in the tumors. He considered it to be one of carcinoma with abnormal cell formation and compared it with Risel's <sup>11</sup> and Davidsohn's <sup>12</sup> cases of carcinoma of the stomach with chorionepithelioma-like metastases. The author's illustrations are of no assistance concerning the origin of the tumor. The probability of incomplete examination of the testes must be borne in mind and this case put on the "doubtful" list.

Blecher and Martius' case <sup>13</sup> was that of a 21 year old male in whom cystoscopy revealed a small tumor on the anterior and superior walls of the right side of the bladder. At operation it was found to be intramural, well circumscribed and completely covered

by mucous membrane. On section it was composed of a number of tumor nodules which showed areas of hemorrhage. Microscopically the tumor failed to show the typical combination of Langhans' and syncytial cells, but instead areas of spindle shaped cells and others with dense medullary columns. Still other areas showed a syncytial arrangement with loss of cell boundaries, giant cells, and also vacuolated cells. The tumor invaded the lymphatics. The testes appeared normal. The patient was free of recurrence 1 year after operation. The authors could not arrive at any definite diagnosis but referred to the similarity to chorionepithelioma on the one hand and "endothelioma" on the other. The author's description and illustrations rather suggest a myosarcoma of the bladder. It certainly cannot be accepted unreservedly as a chorionepithelioma, particularly since the patient was still alive and well 1 year after simple resection of the tumor. The lack of an autopsy follow-up affects the value of this case.

Charkviani<sup>14</sup> reported a case in a 32 year old female who had come to the clinic complaining of vaginal bleeding. A hydatid mole was discovered and the uterus was removed. Subsequently the patient returned and a necrotic tumor of the bladder was found. A tumor nodule was also found in the vagina and was diagnosed as chorionepithelioma on biopsy. X-radiation was applied and the patient improved and was well a year later. Obviously in the presence of the hydatid mole in the uterus the diagnosis of the tumor of the bladder as a primary chorionepithelioma is not justifiable. Furthermore, no report is given of a biopsy of the tumor of the bladder.

Pazourek<sup>15</sup> published a case which apparently was a primary tumor of the bladder, but here again unreserved acceptance is not forthcoming. The patient was a 26 year old married female. A tumor situated on the left bladder wall just above the ureteral orifice on that side was removed by partial resection of the bladder. The author claimed the microscopic appearance was typical of chorionepithelioma but his illustrations do not lend conviction to this conclusion. A curettage was performed soon after resection of the bladder and showed only atrophic endometrium. Pazourek then concluded he was dealing with a primary chorionepithelioma of the bladder which had developed in a "kyste hydatique." The patient was perfectly well 7 months after operation.

From the analysis of the cases reviewed above it is obvious that we are left with only 1 acceptable case of primary chorionepithelioma of the bladder, the 4 others reported in the literature being either too vague in their descriptions and illustrations or obviously not even primary tumors of the bladder. It is important to note that none of these cases had hormonal studies.

Reports of hormone studies in cases of chorionepithelioma in the male are few. Such studies were first carried out by Heidrich, Fels and Mathias in 1930.<sup>16</sup> In our case the rabbit test for gonadotropic hormone in the urine was strongly positive, the test being positive with 1 cc. of urine. In normal pregnancy 20 cc. of urine are customarily necessary to obtain a positive reaction. The rat test gave a positive reaction with 0.1 cc. of urine which is equivalent to 10,000 rat units per liter, which in turn corresponds to the titer usually found in pregnancy. Owen and Cutler<sup>17</sup> consider any reaction from 10,000 to 125,000 m.u. or more per liter in the male as indicative of chorionepithelioma. Belt<sup>18</sup> says that the hormone output is in proportion to the extent and growth of the metastases and that in cases where the metastases are predominantly hemorrhagic in character few hormone active and stimulating cells may be left. The failure to extract gonadotropic or estrogenic substance from the pulmonary metastases in our case may possibly be explained by the partial hemorrhagic necrosis of the tumor nodules. No estrogenic hormone was found in the urine.

Although the morphology of the tumor in our case is characteristically that of a chorionepithelioma, the demonstration of biological activity, as evidenced by the excretion of large amounts of gonadotropic hormone in the urine, leaves no doubt as to its true nature.

Chorionepithelioma arising either retroperitoneally or within the mediastinum is said to have its origin either in germinal rests<sup>7, 19, 20</sup> (*i.e.* the plica urogenitale), or in malignant transformations, as well as in unilateral development of teratomas.<sup>21, 22, 23</sup> Accordingly, its occurrence retroperitoneally or in the mediastinum can be well understood, but its origin in the bladder remains unexplained. For teratomas arising in the bladder the theory has been suggested that they are derived from "dysontogenetic rests of the dorsal mesodermal segments which are carried down to the bladder anlage by the caudally growing



wolffian duct.”<sup>24</sup> Therefore a chorionepithelioma arising in a teratoma of the bladder would have an acceptable mode of development. But when a chorionepithelioma containing no teratomatous elements arises in this region the explanation of its origin is not so simple unless one assumes its origin to be from a teratoma which has developed unilaterally.

Although teratomatous elements were not found in the sections of the primary tumor in the bladder in the case reported here, it cannot be stated definitely that the tumor did not have its origin in a teratoma because, as Kantrowitz<sup>2</sup> has pointed out, the remnants of such elements may be so few and so well localized as to defy their discovery except by the examination of serial sections through the entire tumor. Recalling the size of the neoplasm in this case such an enterprise would be impossible from a practical point of view. In the cases reported in the literature the metastases from ectopic chorionepitheliomas arising in teratomas almost invariably showed no teratomatous elements—only chorionepithelioma. The metastases in our case were of a similar nature.

Although not as striking as in the case of Kantrowitz,<sup>2</sup> nodular hyperplasia of the interstitial cells of the testes was quite evident. A similar finding has been recorded by Arendt<sup>6</sup> and by Heaney.<sup>1</sup> Its significance is unknown.

That certain specific changes occur in the pituitary during pregnancy are well known. In addition to the usual three types of cells in the anterior lobe a cell is present which has certain of the characteristics of both the eosinophil and the basophil, but which differs from them in the size of the cell, the position of the nucleus and the staining qualities. This is the so-called “pregnancy cell” which has its origin in the chromophobe cells of the anterior lobe. The chromophobe cells are transformed into large cells, the cytoplasm of which is filled with fine dust-like granules which stain pink with eosin, and the nuclei are irregular and vesicular. The size of the granules may be contrasted with the much coarser granules found within the cytoplasm of the eosinophils. Actually neither the normally predominant eosinophils nor the basophils vary in number during pregnancy, but an apparent alteration in proportions is brought about by the great increase in modified chromophobe or pregnancy cells. It is noteworthy that changes

similar to those found in pregnancy are also found frequently in carcinoma, particularly the cases with extensive metastases to the liver.<sup>25</sup>

The appearance of the pituitary in pregnancy, as noted above, has also been described in cases of chorionepithelioma in the male.<sup>26-36</sup> However, the number of reports containing descriptions of the pituitary are relatively few and among these are diverse findings. While the majority of authors describe various degrees of "pregnancy change," some describe a less distinctive change and one, in fact, reports no change at all. Stöckl's case<sup>31</sup> goes to the other extreme in that he finds a marked increase and predominance of basophilic cells. In our case the proportions of basophils to eosinophils appeared to be within normal limits. However, scattered throughout the anterior lobe were a moderate number of large, irregularly shaped cells which were distinctly reminiscent of "pregnancy cells." Their cytoplasm was filled with fine granules which showed an affinity for the acid stains. Many of the basophils and eosinophils showed vacuole formation in the cytoplasm.

#### SUMMARY AND CONCLUSIONS

A primary chorionepithelioma of the urinary bladder in a 70 year old male is reported, the 2nd case to appear in the literature.

The testes, vas deferens, seminal vesicles and prostate showed no tumor involvement. Sections from 1 to 2 mm. in thickness were made throughout the entire thickness of both testes and microscopic slides were made from each block. No tumor nodules were found. Slight tubular atrophy and nodular interstitial cell hyperplasia were present.

Sections through the primary tumor in the bladder failed to reveal any teratomatous elements. However, the possibility of the unilateral development of this tumor from a teratoma cannot be disproved.

The incomplete "pregnancy" reaction in the pituitary as well as metastatic involvement of the same organ is discussed.

Both the rabbit and the rat tests for gonadotropic hormone were significantly positive in the urine. Tests for estrogenic hormone were negative. Extracts of tumor tissue from the lungs failed to show either gonadotropic or estrogenic activity.

The origin of the tumor in dysontogenetic rests of the dorsal mesodermal segments is suggested.

NOTE: The author wishes to thank Dr. Robert T. Frank for his kindness in performing the hormonal studies in this case.

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## DESCRIPTION OF PLATES

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### PLATE 113

- FIG. 1. Urinary bladder opened anteriorly to show the primary tumor mass involving the entire right lateral wall and a greater portion of the posterior wall. The tumor mass extends from the neck of the bladder far into the fundal region. Areas of hemorrhage are seen in the cross section of the right lateral wall in the fundal region.
- FIG. 2. The lungs have been hemisected and the cut surfaces of the anterior halves are shown. Note the numerous, large, well circumscribed projecting tumor nodules scattered throughout the parenchyma.



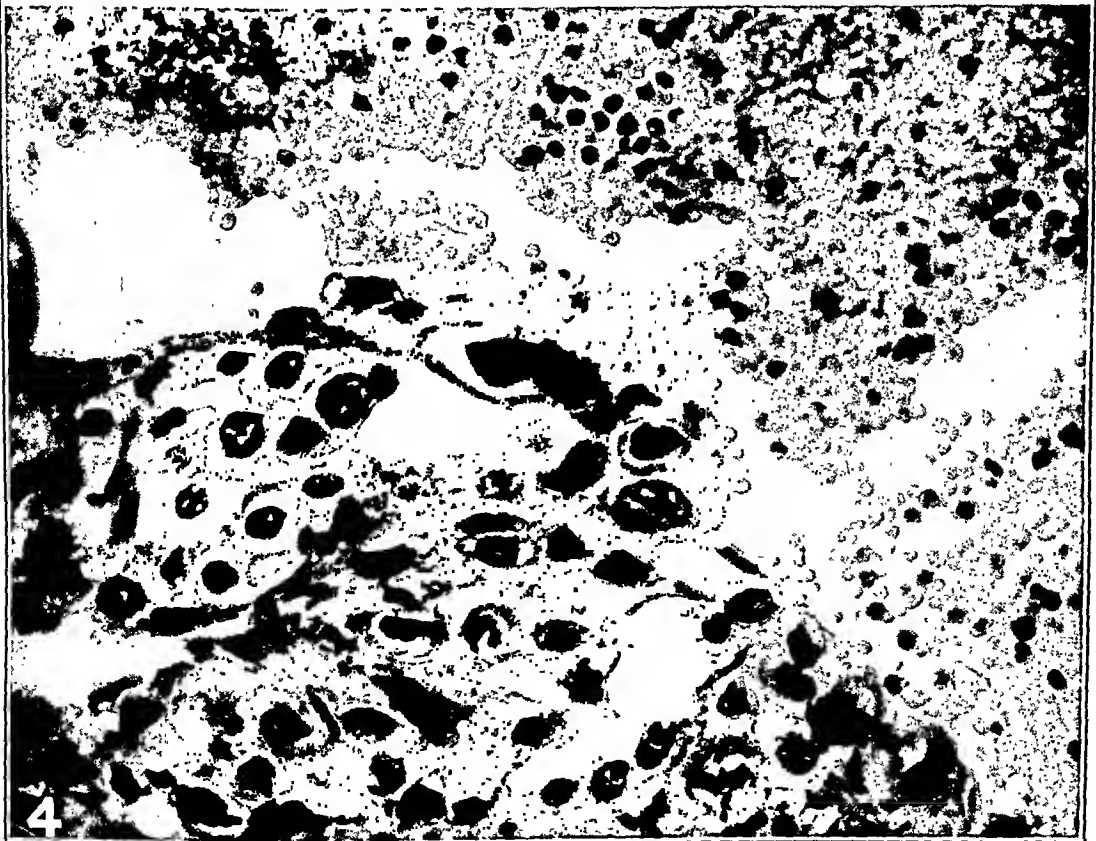
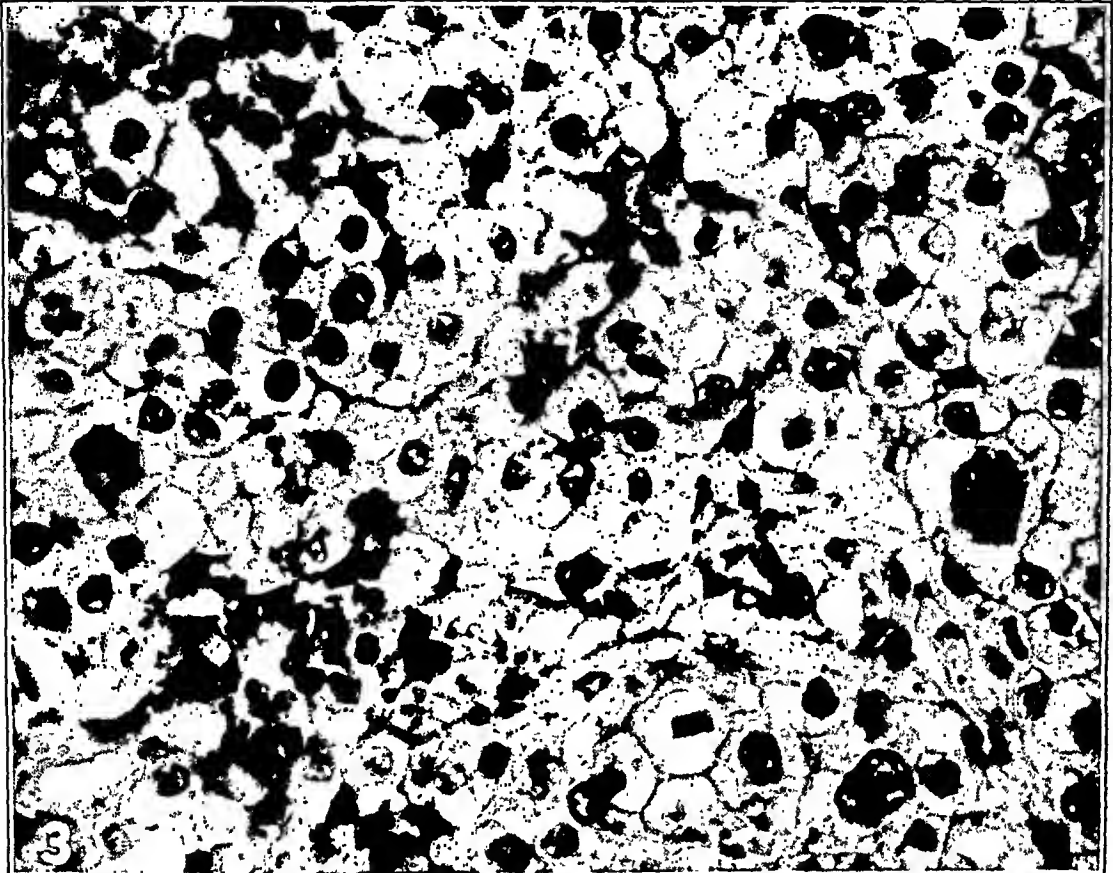
PLATE 114

FIG. 3. Microphotograph of the primary tumor of the bladder showing a representative area composed of Langhans' cells.  $\times 620$ .

FIG. 4. Microphotograph of primary bladder tumor showing nests of syncytial cells and an area of hemorrhage.  $\times 620$ .









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## EXPERIMENTAL PNEUMONIA PRODUCED BY TYPHUS RICKETTSIAE \*

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After Prowazek and da Rocha-Lima demonstrated that the organism of typhus fever multiplied within the cells of the intestinal tract of infected lice, it was assumed that Rickettsiae were obligate parasites of certain animal cells. This was confirmed by Wolbach and coworkers<sup>1</sup> who observed intracellular Rickettsiae in the endothelium of the blood vessels of humans and animals infected with typhus. A few years later Mooser<sup>2</sup> discovered intracellular organisms in the mesothelial cells of the tunica vaginalis of typhus infected guinea pigs, and Zinsser and Castaneda<sup>3</sup> cultivated Rickettsiae in large numbers in the peritoneum of rats where the organisms multiplied readily within the serosal cells. Okamoto<sup>4</sup> reported that he had observed Rickettsiae in the alveolar cells of the lungs of mice infected by the intraperitoneal route. Recently Hitz,<sup>5</sup> in our laboratory, succeeded in cultivating Rickettsiae in minced guinea pig lung suspended in an ascitic-serum mixture. The cultures did not grow as readily as those made from the tunica vaginalis, but his findings corroborate indirectly those of Okamoto. He observed also, in cultures made from the tunica vaginalis, typical Mooser cells in close proximity to muscle fibers suggesting a relationship with the connective tissue sheaths, although the true nature of these cells has not been determined.

In a recent preliminary report<sup>6</sup> we showed that mice and rats could be infected by the intranasal route and that a considerable growth of Rickettsiae could be obtained in the lung. It was also stated that the lining of the bronchi was found to be parasitized with intracellular bodies. The cells were infected in such a manner that the epithelium resembled the gastro-intestinal tract of typhus infected lice.

The various types of cells in which Rickettsiae have been found

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show that the cellular requirements of the agent of typhus are not as limited as we had assumed. In this report we wish to confirm our first experiments and give further information concerning these observations.

### MATERIAL AND METHODS

The strain of typhus used for the present experiments was our "L" orchitic strain isolated from a case of typhus in the General Hospital of Mexico City in 1936. This strain has been transferred over 150 times in male guinea pigs which have constantly shown the typical scrotal reaction.

Adult mice and young white rats were inoculated by the intranasal route by means of a 1 cc. pipette applied to the nostrils, administering very slowly a total of 0.2 cc. to the mice and 0.4 cc. or a little more to the rats. For inoculation the animals were anesthetized with ether and the anesthesia was repeated as necessary until the whole dose of inoculum was given.

The inoculum prepared from the guinea pig material was obtained by washing the tunica vaginalis with 1 cc. of an isotonic sodium citrate solution for each guinea pig used. The washings were centrifuged at low speed in order to remove gross particles. The inoculum prepared from the infected lungs of mice or rats was obtained by grinding the lungs with powdered sterile glass and emulsifying them in saline. The lungs of mice were suspended in 7 cc. and those of rats in 30 cc. each of saline. The emulsions were then centrifuged at low speed to remove the particles of glass.

Rabbits of various sizes were anesthetized with Dial (Ciba) by the intraperitoneal route in amounts of 0.55 cc. per kilo of body weight, and others with ether. When completely anesthetized 8 to 10 cc. of the inoculum was injected directly into the trachea. When desired, the body temperature of the inoculated rabbits was kept below 37° C. by repeating the injection of Dial. Three or 4 doses, each containing 75 per cent of the original dose, applied at convenient intervals of time, were sufficient to keep the animals 72 hours or more under narcosis and at low body temperature. As stated elsewhere,<sup>7</sup> low body temperature favors the growth of typhus Rickettsiae.

The lungs of the animals found dead or killed at various intervals of time after inoculation were placed in sterile Petri dishes

and plain or blood agar slants were smeared with small pieces of tissue. Smears and impressions on slides were made for direct examination and fragments of the lung were fixed for histological study.

The smears were stained by Giemsa's method and by our methylene blue-safranin stain. Tissues were fixed in Regaud's solution (potassium bichromate 3 per cent, sodium sulphate 1 per cent, and formalin 10 per cent). Sections were stained with a modification of one of Pappenheim's methods, recommended by Hitz,<sup>5</sup> as follows:

#### SOLUTION A

Distilled water . . . . .	100 cc.
Glacial acetic acid . . . . .	1 drop
May-Grünwald stain . . . . .	20 cc.

#### SOLUTION B

Distilled water . . . . .	100 cc.
Glacial acetic acid . . . . .	1 drop
Giemsa's stain . . . . .	5 cc.

After treating the sections with Solution A for 15 minutes, transfer without washing into Solution B and leave for 30 minutes to 1 hour. Both solutions have a better action at 37° C. Dehydrate rapidly with absolute alcohol and after clearing in xylol mount in cedar oil. This seems to be the simplest way of staining Rickettsiae in sections.

### EXPERIMENTAL

The inoculation into mice of washings from the tunica vaginalis of typhus infected guinea pigs by the intranasal route gives rise to fatal results in a large percentage of the animals. The mice die usually about 96 hours after inoculation and show pneumonic lesions characterized by considerable hyperemia and hemorrhage of the lungs, which usually become completely involved. The affected lobes of the lungs resemble liver or spleen. The non-affected tissue shows a compensatory emphysema. If the lungs are left in a Petri dish for a few minutes there is an exudation of blood which soon coagulates.

Microscopic examination of the lungs shows that the capillaries are filled with blood and many extravasated red cells have invaded

the alveoli. There is also an infiltration by polymorphonuclear leukocytes, which are present in large numbers but do not suggest pus formation. Many leukocytes show various degrees of necrobiosis, mainly pyknosis. The cellular degeneration seems to involve the cells of the alveoli, bronchi and capillaries. The cytoplasm of many cells is swollen by considerable numbers of small organisms. Many of these cells appear to belong to the blood capillaries, but the epithelium of the bronchi is also found to be infected with the same parasite, presenting an appearance similar to that of the intestinal tract of typhus infected lice. The infected bronchial cells, as well as those scattered in the lungs, resemble the so-called Mooser cells, so characteristic of the lesions of the tunica vaginalis and the peritoneal infection of X-rayed, typhus infected rats. Extracellular organisms may also be seen but are not very numerous. In some animals ordinary bacteria are also present. Smears or impressions made from the lungs of infected animals show large numbers of small intracellular and extracellular organisms. Many polymorphonuclear leukocytes have phagocytosed these organisms. The general appearance resembles the smears made from the peritoneal exudate of X-rayed, typhus infected rats.

The inoculation of washings from the tunica vaginalis of infected guinea pigs into rats of various sizes has given very irregular results. Only a few animals have developed lesions in the lungs after inoculation. The microscopic appearance is similar to that observed in mice and Mooser cells are easily found.

#### TRANSFER OF THE LUNG INFECTION BY MEANS OF A LUNG EMULSION

Rats, mice and rabbits inoculated by the intranasal route with emulsions of the lung from infected animals died within 72 to 96 hours after inoculation with extensive lesions in the lung identical with those we have described.

It has been possible to transfer the infection from rat to rat for several generations with the same characteristic hemorrhagic lesions developing in the lungs. In one instance a "lung strain" was obtained from a rat which developed lesions after infection with washings from the tunica vaginalis of infected guinea pigs. This strain which killed the animals within 4 to 6 days was unfortunately lost on the 7th transfer.

## CULTURES ON PLAIN OR BLOOD AGAR FROM INFECTED LUNGS

Many attempts to cultivate ordinary bacteria were made, using as a medium plain or blood agar slants. For this purpose small pieces of lung were cut as far as possible from the large air ducts and were smeared on the slants. The mice infected by washings from the tunica vaginalis of infected guinea pigs showed few or no colonies on the slants after 48 hours incubation. In rats inoculated with emulsions from the lungs of mice, contaminating organisms were rarely found, but in transfers from rat to rat ordinary bacteria, usually Gram-negative, were cultivated on various occasions. These organisms were tested with typhus serum and did not give the agglutination reaction. Emulsions of the Gram-negative bacilli were inoculated into guinea pigs intraperitoneally and produced a peritoneal infection with death of the animal in from 48 hours to 8 days. The exudate showed the injected organisms, but Mooser cells were not found. The inoculation of large doses of the same Gram-negative bacteria into rats by the intranasal route failed to produce the hemorrhagic pneumonia shown by those inoculated with typhus material or with emulsion from lung transfers.

## RESISTANCE OF RATS AND MICE TO A SECOND INTRANASAL INOCULATION

The rats and mice which survived inoculation with either washings from the tunica vaginalis or emulsion of the lungs from infected mice were reinoculated 10 to 15 days later with an emulsion of the lungs from an infected mouse by the intranasal route. These animals survived the test.

## IDENTIFICATION OF THE ETIOLOGICAL AGENT PRODUCING HEMORRHAGIC PNEUMONIA IN MICE, RATS AND RABBITS INOCULATED WITH TYPHUS MATERIAL

Several guinea pigs were inoculated intraperitoneally with emulsions made from lungs showing typical hemorrhagic lesions and many Mooser cells. The material was obtained from rats infected with virus from the 3rd to the 7th transfer from lung to lung. As a control, typhus immune guinea pigs were injected with the same material.

In one instance both normal and immune guinea pigs died on the 3rd or 4th day after inoculation with a peritoneal infection by a Gram-negative bacillus similar to that isolated on agar cultures. No bodies resembling *Rickettsiae* were observed.

Two normal guinea pigs showed fever and swelling on the 4th day after inoculation and one died from peritonitis on the 6th day. The other animal was killed and material was transferred into another guinea pig. Smears from the tunica vaginalis showed typical Mooser cells, but a few bacteria were also seen. The guinea pigs inoculated with this material developed a peritoneal infection with a Gram-negative organism. The typhus immune guinea pig controls also died with peritonitis.

The inoculation of emulsion of the lung of the 5th generation from rat to rat produced in a normal guinea pig typical fever and swelling without intercurrent infection, while the typhus immune controls showed no fever or swelling. These animals were tested later with the "L" strain and showed no reaction at all.

A normal and an immune guinea pig were inoculated with an emulsion of the brain from a rat which was previously inoculated with washings from the tunica vaginalis of guinea pigs inoculated with material from the lung. The normal animal developed typical fever and swelling and was found to be immune when reinoculated with the "L" typhus strain. The immune guinea pig showed no reaction at all.

Guinea pigs were vaccinated with 2 doses of 1 cc. each, given subcutaneously, of an emulsion of organisms obtained from the lungs of infected rats. The suspensions made in formalinized saline were purified by fractional centrifugation and contained  $3 \times 10^9$  organisms per cc. When the guinea pigs were tested 15 days after the first vaccination they were found to be immune to the "L" strain.

Purified emulsions of organisms found in the lungs of infected rats were submitted to microscopic agglutination tests. These were made by mixing a droplet of serum with a drop of the emulsion and adding a little methylene blue. The mixtures were placed in a hanging drop preparation and observed under the No. 40 objective. The mixtures containing normal human or guinea pig serum showed no agglutination. Those containing human convalescent typhus serum or immune guinea pig serum were aggluti-

nated within a short time. Care was taken to use the serums of guinea pigs bled before and at various intervals of time after the inoculation with orchitic typhus.

To this data we may add the further information that guinea pigs inoculated with minute amounts of emulsion of the lungs from mice inoculated with washings from the tunica vaginalis of guinea pigs, and from rats infected from such mice, invariably develop typical typhus infection.

From these various experiments we conclude that the organisms found in the lungs of rats and mice infected with typhus material and transmitted by emulsions of the lung to other animals are *Rickettsiae prowazeki* which grow in great numbers in the cells of the bronchi, the alveoli and the endothelium of the capillaries. The contaminating organisms are easily detected by cultivation on agar slants and inoculation into animals. These contaminants have a tendency to increase in proportion with the transfers from rat to rat and may finally predominate in the lungs, but are rare in mice infected with material from guinea pigs and in rats inoculated with emulsions from the lungs of mice.

#### PRODUCTION OF LARGE QUANTITIES OF RICKETTSIA BODIES FROM INFECTED LUNGS

When a mouse is inoculated with washings from the tunica vaginalis of typhus infected guinea pigs sufficient amounts of *Rickettsiae* are produced to infect 15 medium sized rats. Whenever the infection is successful the mice die 96 hours after inoculation and the rats infected with emulsion from the lungs die with great regularity on the 3rd day after inoculation.

Rabbits were anesthetized with Dial and inoculated with an emulsion of the lungs from infected rats. Each rabbit received about one-third of a whole lung. The animals were kept at low body temperatures, which is essential to obtain abundant growth of *Rickettsiae*.<sup>7</sup> Rabbits inoculated by the intratracheal route but not submitted to a depression of temperature, developed a fatal disease with considerable involvement of the lungs and showed *Rickettsiae* in large numbers, but the yield was negligible compared to that obtained from animals subjected to a low body temperature.

From the lungs of medium sized rats we obtained, after grind-



ing and purifying by fractional centrifugation to remove foreign particles, about 10 gm. of packed *Rickettsiae* per 100 animals. This may be diluted to 5 liters or more to obtain a concentration of *Rickettsia* bodies suitable for vaccination. Two technicians may easily inoculate 3 lots of 100 rats a week to obtain about 15 liters of vaccine.

We have not so far calculated the production that may be obtained from rabbits kept at low body temperatures by continued narcosis with Dial, but roughly one may estimate that as much vaccine can be obtained from 1 rabbit as may be obtained from 10 rats.

The various methods of production of the Mexican vaccine first prepared by Zinsser and Castaneda enable us to obtain sufficient amounts of formalin-killed *Rickettsiae* which may be used advantageously as a prophylactic means against typhus.

#### SUMMARY

The intranasal inoculation of mice and rats with typhus virus (orchitic variety) has given rise to hemorrhagic lesions of the lungs which kill mice in 96 hours and rats in 72 hours each. The lungs show in sections and smears considerable numbers of *Rickettsia* bodies which have been obtained in pure suspension by grinding and fractional centrifugation. Rabbits have also been infected by the intratracheal route with or without forcing down the body temperature. The animals develop hemorrhagic pneumonia, and *Rickettsiae* are present in large numbers in smears and in sections of the lungs, but the animals subjected to a low body temperature produce greater quantities of *Rickettsia* bodies. These rabbits die in from 48 to 96 hours after inoculation. The rabbits not submitted to a low body temperature die after a longer period of time and show lesions of the lungs which are more extensive but which contain fewer *Rickettsiae*.

To produce massive infection of the lungs it is necessary to inoculate considerable numbers of *Rickettsiae*.

This method of cultivating *Rickettsiae* has proved very useful for obtaining typhus vaccine for practical purposes.

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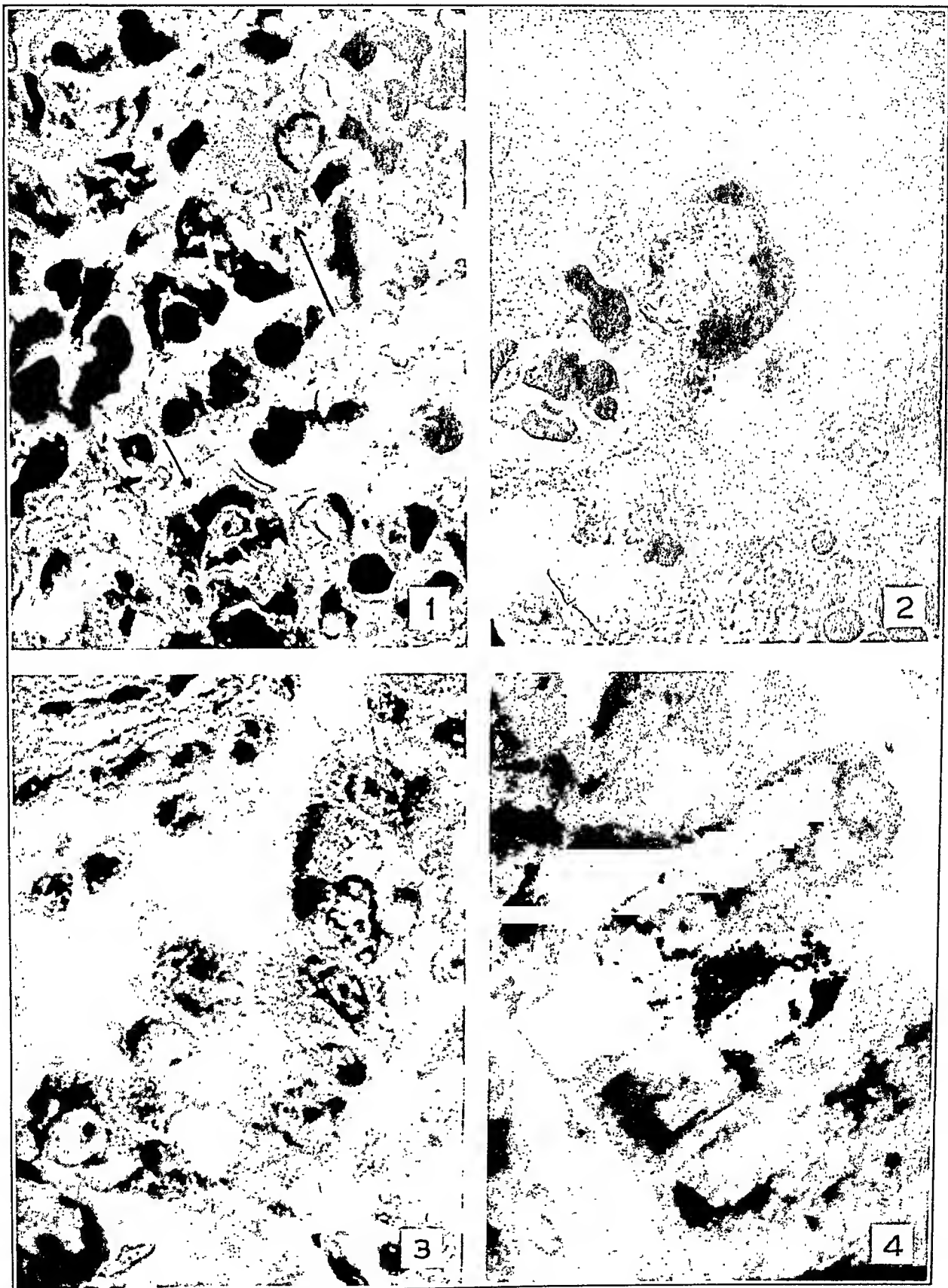
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## DESCRIPTION OF PLATE

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### PLATE 77

- FIG. 1. Lung of rat infected with typhus Rickettsiae showing two Mooser cells indicated by the arrows.
- FIG. 2. Parasitized cells from the lung of a rat, apparently from a capillary.
- FIG. 3. Section showing the bronchial epithelium parasitized with Rickettsiae.
- FIG. 4. Higher power of Fig. 3 showing bronchial cells filled with Rickettsiae.





## HISTOPLASMOSIS IN INFANCY \*

### REPORT OF A CASE

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Histoplasmosis is a rare mycotic infectious disease. Six cases are usually cited in recent texts. A review of the recent literature indicates that 3 additional cases can be added — 1 by Hansmann and Schenken,<sup>1</sup> 1 by Phelps and Mallory,<sup>2</sup> and 1 by Müller.<sup>3</sup> The following case which came to our attention makes the 10th case of human histoplasmosis reported to date.

### REPORT OF CASE

*Clinical History:* A female infant was delivered by cesarean section in Detroit. Shortly after birth the infant was removed to Missouri for a time. After returning to Detroit she developed a chronic respiratory condition characterized by intermittent paroxysms of coughing which was diagnosed as pertussis. The child developed a left otitis media followed by a persistent serosanguineous discharge. The respiratory symptoms persisted for 9 weeks, at which time the mother became alarmed because of the child's pallor and the enlarging abdomen. The infant was hospitalized and the anemia was interpreted as secondary to some infection. A roentgenogram of the thorax showed indefinite peribronchial infiltration near the hilum of each lung.

The infant was removed to another institution where she was hospitalized for 57 days. Here she ran a continuous febrile course and the paroxysms of coughing continued. The abdominal enlargement increased and was found to be due to a hepatosplenomegaly.

Blood studies showed a constant low erythrocyte count averaging 2,000,000 per cmm., and hemoglobin values which averaged slightly less than 50 per cent. There was a constant leukopenia, the leukocyte count averaging 1500 cells per cmm. The differential counts showed the neutrophilic leukocytes to average between 45 and 55 per cent, lymphocytes 40 and 60 per cent, and monocytes 2 and 12 per cent. Numerous normoblasts were present. This blood picture persisted in spite of repeated small transfusions of blood and therapy with liver extract and pentnucleotide.

On the basis of a clinical diagnosis of splenic anemia, splenectomy was performed. Because of the poor physical state of the patient no additional exploration was attempted.

Subsequent to the splenectomy there was definite improvement in the blood picture: the erythrocyte count rose steadily to 3,500,000, the hemoglobin rose to 60 per cent, and the leukocyte count rose to 5500. However, the fever, weakness and abdominal enlargement persisted.

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The child died at the age of 8 months, 3 weeks after the splenectomy and about 4 months after the onset of symptoms. At no time was jaundice noted. Slight edema of the lower extremities was present at intervals. During the last 10 days of life some stiffness of the neck without rigidity was present, but the Kernig test was negative. The spinal fluid was under slightly increased pressure and contained 1 lymphocyte per cmm.

### COMMENT

The exact nature of the disease was not recognized until several months after the death of the child when the pathological condition present was identified by a histological examination of the spleen. Permission for an autopsy was refused.

The spleen removed at operation weighed 159 gm. The organic form was preserved and the capsule was thin. On section the pulp was rather firm in consistence and a grayish pink in color. The malpighian corpuscles were not prominent.

The most striking pathological feature seen on microscopic examination was the tremendous proliferation of reticuloendothelial cells (Fig. 1). The pulp was crowded with great numbers of large cells of the macrophage type, many of which contained clusters of round or slightly oval bodies (Figs. 2 and 3). These bodies were made up of a thick, clear, non-chromatic capsule surrounding a finely granular cytoplasm. The chromatin was ordinarily aggregated at one pole of the cell and was often arranged as a crescent or occasionally as a compact dot located near the capsule of the cell. A blepharoblast was not demonstrable in these parasites, a feature differentiating this organism from the Leishman-Donovan body. The number of parasites within individual macrophages varied from a few to as many as 25. Frequently they occurred in clusters, suggesting a mulberry arrangement with no remnant of the macrophage to be seen. The splenic blood sinusoids contained a large number of macrophages, some of which had phagocytosed variable numbers of parasites. Most of the red blood corpuscles in the splenic pulp were hemolyzed, and phagocytosis of blood pigment was prominent. Some macrophages contained both parasites and blood pigment.

Subsequent to the discovery of the parasites in sections of the spleen two blood films which had been prepared during the last week of illness were reexamined. In these blood smears the parasites were found phagocytosed in large mononuclear cells (Fig. 4) and occasionally in neutrophilic polymorphonuclear leukocytes.

## DISCUSSION

All of the reported cases of histoplasmosis have terminated fatally; in only 2 cases was the nature of the disease recognized before death so that cultural studies could be made. We are indebted to DeMonbreun<sup>4</sup> for his classical study of the infective agent isolated from the case reported by Dodd and Tompkins.<sup>5</sup>

As yet, mycologists do not agree as to the exact classification of this yeast-like fungus. If we accept the observations of Moore,<sup>6</sup> which are supported by Dodge,<sup>7</sup> the fungus recovered from the case reported by Dodd and Tompkins and that from Hansmann and Schenken's case belongs to the genus *Histoplasma* (*Posadasia*) of the family *Coccidioideaceae*. Two species of the genus *Histoplasma* are recognized, *Histoplasma capsulatum* and *Histoplasma pyriforme*. DeMonbreun and the Italian workers, Redaelli and Cifarri,<sup>8</sup> do not believe that the *Histoplasma* fungus produces asci and the latter two workers prefer to group *Histoplasma* with *Cryptococcus*.

The epidemiology and pathogenesis of histoplasmosis await further study. The occurrence of respiratory disturbances in 6 of the reported cases suggests that the portal of entry into the human host is most likely through the respiratory system. This finds additional support from the early clinical picture in our case. The infant exhibited early and persistent respiratory symptoms in the nature of intermittent paroxysms of coughing which simulated pertussis. In the case reported by Dodd and Tompkins similar respiratory symptoms were present. On the other hand, the prominent intestinal disturbances in Müller's case and the dominance of granulomatous lesions in the intestine and mesenteric lymph nodes in the case reported by Crumrine and Kessel,<sup>9</sup> suggest that the portal of entry may at times be through the intestinal mucosa. The association of intestinal ulcers in 2 of Darling's cases,<sup>10</sup> and also in Müller's case, tends to support this view. Again, it is possible that the portal of entry may rarely be through the skin. In the case reported by Hansmann and Schenken there was a protracted, chronic papulopustular dermatitis from which *Histoplasma* were recovered.

Irregular fever, weakness, anemia, leukopenia and splenomegaly are the features usually described in the clinical course of the disease.



Pathologically the most prominent feature is the marked reticuloendothelial proliferation, especially in the spleen, lymph nodes, liver and bone marrow. Large numbers of macrophages are formed which engulf the parasites. There is a tendency for pseudotubercles to be produced in the lungs, and in 3 of the reported cases superficial ulcers of the intestine were observed.

Case reports of histoplasmosis indicate that the parasite may be found in the epithelial cells of the intestine and the bronchi, and even in the cortical cells of the adrenal gland. In the adrenal, caseous lesions simulating tuberculosis may be produced (Hansmann and Schenken<sup>1</sup>).

The accumulated data, however, indicate that the classical features of the disease are subject to considerable variation. Thus anemia was not a feature of Hansmann and Schenken's case, and leukocytosis was present in the cases reported by Phelps and Mallory, Hansmann and Schenken, and particularly in that reported by Dodd and Tompkins. Splenomegaly was absent in 2 cases, those of Hansmann and Schenken, and Crumrine and Kessel. The one feature common to all was the presence of the characteristic clusters of phagocytosed yeast-like fungi in reticuloendothelial cells of various organs.

Redaelli<sup>11</sup> worked with laboratory animals which had been inoculated with cultures of the organisms recovered from the case reported by Hansmann and Schenken. His experimental studies demonstrate an extreme degree of proliferation of reticuloendothelial cells in the lymph nodes, spleen and bone marrow, and an active phagocytosis of the organism by macrophages. In the early course of the experimental cases phagocytic activity was pronounced. However, sooner or later there appeared to be a collapse of this phagocytic power, and in those animals that survived infection with the saprophytic form of the fungus there remained continued impairment of phagocytic function as determined by the Congo red test.

#### SUMMARY

A fatal case of infantile histoplasmosis is reported. Apparently this is the 10th case of human histoplasmosis to be recorded, and the 2nd case observed in an infant.

The clinical manifestations were chronic paroxysmal cough,

anemia, leukopenia, continuous fever, weakness and hepatosplenomegaly.

Of the organs, only the surgically removed spleen was available for study. This showed a marked proliferation of reticuloendothelial cells, many of which contained the fungus.

Phagocytic cells laden with parasites were found in blood smears from the circulating blood.

The Histoplasma is a fungus, the pathogenic form of which resembles yeast. The saprophytic form is a myceliate spore-bearing fungus whose exact taxonomic position is still in question.

Knowledge regarding the epidemiology and pathogenesis of histoplasmosis is incomplete.

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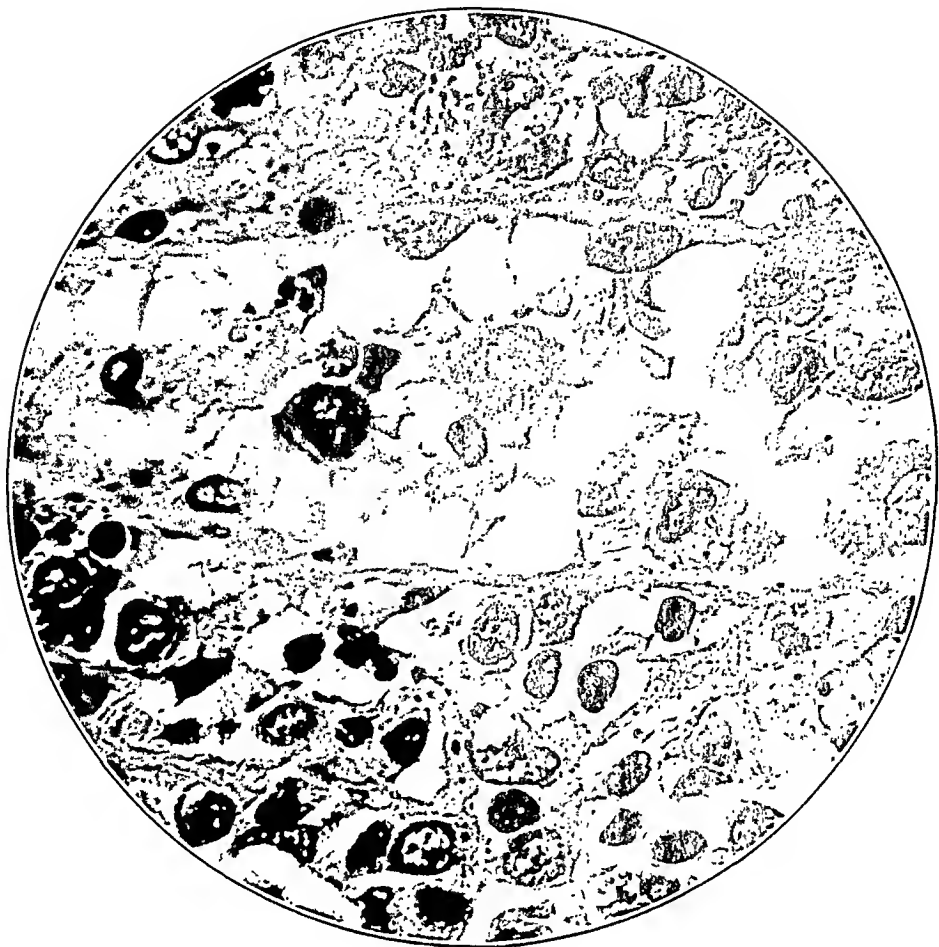
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## DESCRIPTION OF PLATES

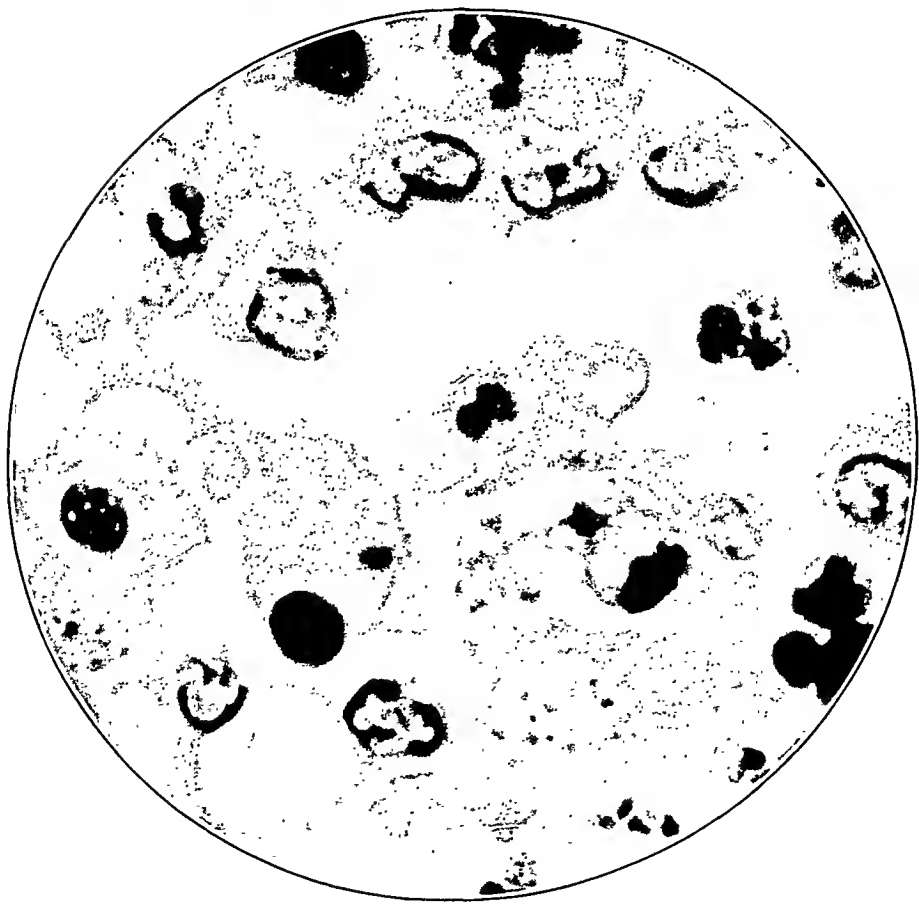
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### PLATE 78

- FIG. 1. A splenic blood sinusoid with surrounding red pulp showing reticulo-endotheliosis. Numerous encapsulated blastospores in small and large clusters are seen. Macrophages are present in the sinusoid, many of which show degenerative changes. Iron hematoxylin and Masson's trichrome lichtgrün stain.  $\times 1000$ .
- FIG. 2. A splenic blood sinusoid containing macrophages. Clusters of *Histoplasma capsulata* are seen in the degenerated pulp cells. Iron hematoxylin and Masson's trichrome lichtgrün stain.  $\times 1400$ .



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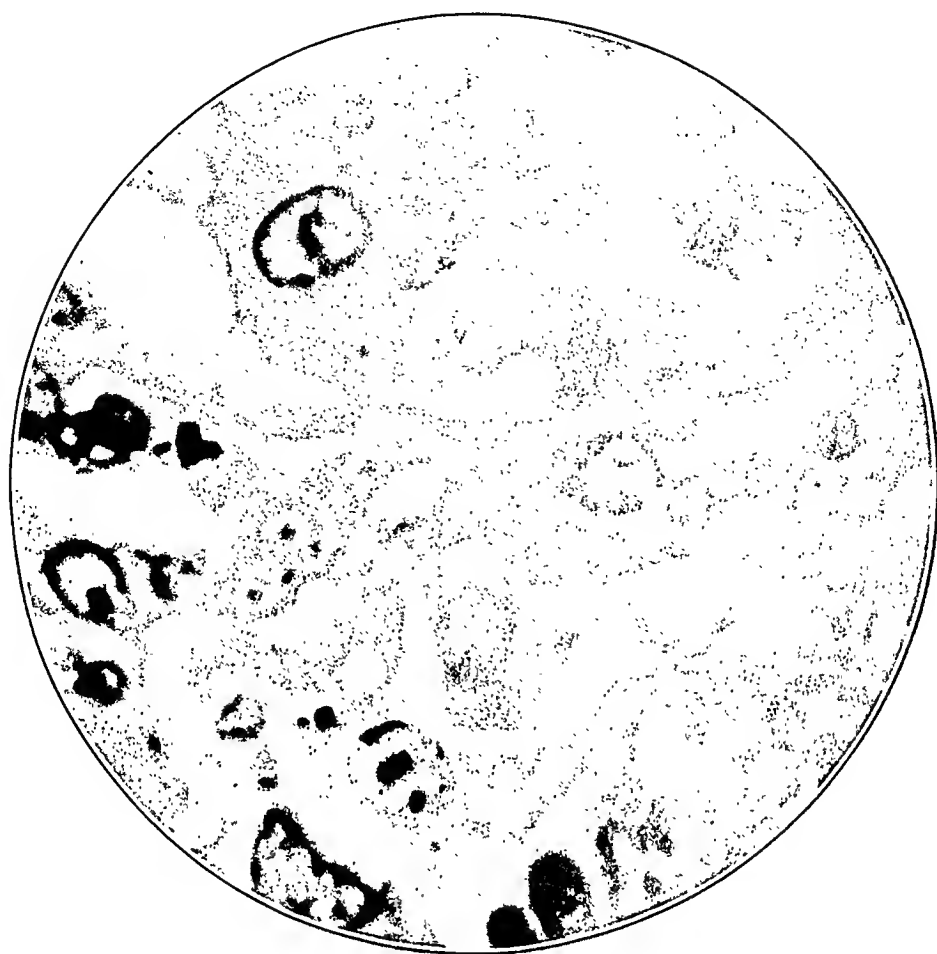


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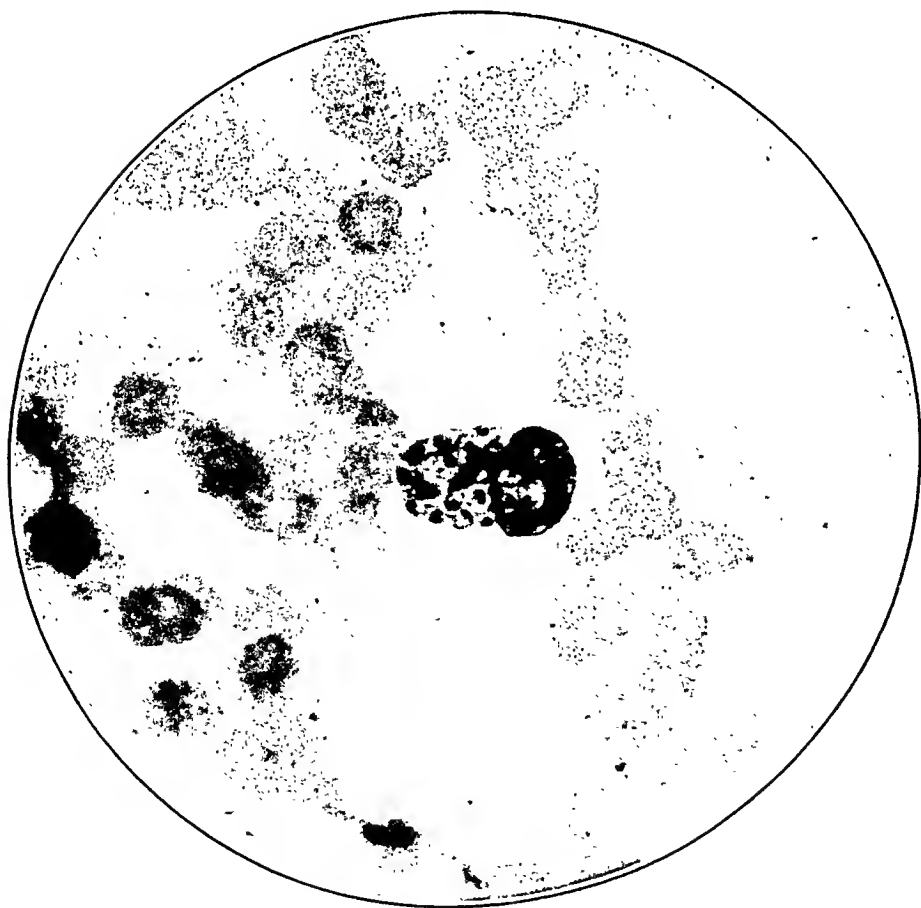
PLATE 79

FIG. 3. A degenerating macrophage in a splenic blood sinusoid is shown on the right. A cluster of *Histoplasma capsulata* is present in a degenerated macrophage in the pulp. Iron hematoxylin and Masson's trichrome lichtgrün stain.  $\times 1400$ .

FIG. 4. A macrophage in the peripheral blood showing a large cluster of *Histoplasma capsulata* in the cytoplasm crowding and deforming the cell nucleus. The typical mulberry-like formation is seen. Wright's blood stain.  $\times 1400$ .



3



4



# MALABSORPTION OF FAT (INTESTINAL LIPODYSTROPHY OF WHIPPLE)\*

## REPORT OF A CASE

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In 1907 Whipple<sup>1</sup> reported a fatal case of "a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues." The patient was a physician, aged 36 years. The clinical course of the disease was characterized by recurring attacks of arthritis, progressive emaciation, enlargement and tenderness of the abdomen and fatty diarrhea. At autopsy neutral fat and fatty acid deposits were found in the intestinal mucosa and in the mesenteric and retroperitoneal lymph nodes. The mesenteric lymph nodes were greatly enlarged, some measuring 3 to 4 cm. in diameter. The cut surfaces of the nodes presented an opaque pale yellow color with almost complete disappearance of the glandular tissue. The retroperitoneal lymph nodes were of similar character. The microscopic appearance of the jejunum, ileum and the glandular lesions may be summarized as follows: The villi were enlarged. The lymphatic channels were dilated and filled with large fatty masses. There were large numbers of "polyblasts and large mononuclear ameboid cells with a pink granular protoplasm. Large 'foam' cells were present. Examination of the lymph glands revealed the process to begin in the sinuses with invasion of the characteristic cells and small irregular fat deposits. The final stage presented a very large gland packed with fat deposits of all sizes and shapes, whose stroma was made up of dense fibrous tissue full of ecchymoses and great numbers of giant and mononuclear cells." Whipple suggested that the term "intestinal lipodystrophy" be given the newly described disease.

Blumgart<sup>2</sup> in 1923 reported 3 fatal cases of malabsorption of fat in adults. All of the cases presented indefinite clinical outlines. The occurrence of fatty stools, great loss of weight and strength,

\* Received for publication April 10, 1939.



and enlarged mesenteric lymph nodes containing fatty substances, suggested a relation to the case described by Whipple. None of Blumgart's patients had arthritis, eosinophilia, purpura or enlarged intestinal villi, but all showed the significant anatomical deposits of fat confined to the intestinal mucosa and the mesenteric lymph nodes. Small granular elevations, gray in color, were present in the mucosa of the small intestines. On microscopic examination these lesions were found to consist of groups of phagocytes containing ingested fat. The phagocytes were large and mononuclear, and contained a foamy reticulated cytoplasm. The mesenteric lymph nodes were noticeably enlarged and hyperplastic and contained similar phagocytes.

Jarcho<sup>3</sup> in 1936 reported a case of steatorrhea with unusual intestinal lesions, which he described as being clinically similar to and anatomically identical with the case reported by Whipple. Jarcho reviewed the cases of Whipple and Blumgart. He classified Blumgart's 2nd case as analogous to the case reported by Whipple. Jarcho stated that these 3 cases were characterized by "unusual and dense deposits of fat throughout the length of the jejunum and ileum and in the mesenteric lymph nodes with concomitant infiltrates of mononuclear cells and giant cells; the latter were frequently found applied to the margins of fatty deposits. There were no morphological changes in the pancreas and there was no evidence of active infection, tuberculous or other."

Boeck, in discussing a paper by Bargaen, Bollman and Kepler<sup>4</sup> on the diarrhea accompanying pancreatic insufficiency, described a case quite suggestive of intestinal lipodystrophy. The patient was a 45 year old physician who had persistent steatorrhea, abdominal distention and a moderate secondary anemia. A laparotomy was done and all the mesenteric glands were found enlarged. Microscopic examination of one of the lymph nodes revealed almost complete replacement of lymphoid tissue with "reticulocyte cells" filled with fat, and deposits of cholesterol crystals. At autopsy fat and cholesterol deposits were also found in the walls of the small intestine, with some mucosal atrophy.

Through the courtesy of Dr. W. B. VandeGrift of the Department of Pathology of The Johns Hopkins University, tissue slides from the following case were compared with those from the cases

of Whipple and Jarcho and the opinion was expressed that the anatomical lesions were of the same basic type.

### REPORT OF CASE

*Clinical History:* A 74 year old retired carpenter was admitted to the Starling-Loving University Hospital with the complaint of an enlarged abdomen and constipation. He had been in good health until about 1 year previous to admission, at which time he became conscious of abdominal discomfort after meals. Because of postprandial discomfort he decreased his food and liquid intake. He lost 35 to 40 pounds in weight during the preceding year. Pitting edema of the extremities, dyspnea on slight exertion and orthopnea developed during the 3 weeks previous to admission. He had had a moderately severe generalized pruritis, most marked in the evening. There was no history of jaundice or of diarrhea. Over a period of 30 days in the hospital 36 stools were passed which varied in frequency from 5 in 1 day to none in 2 days. None were passed during 11 of the 30 days. The stools varied from a large to a moderate amount, were liquid to soft in consistence, and were of a light brown to brown color. Apparently they were not sufficiently unusual to attract clinical attention.

*Physical Examination:* The patient was an emaciated elderly male, moderately orthopneic and dyspneic. The temperature was normal. Superficial lymph nodes were palpable and the cervical lymph nodes were slightly enlarged. The mouth was edentulous and the tongue smooth but not atrophic. Crepitant râles were heard throughout the bases of both lungs. The pulse frequency was 80, the rhythm regular and the volume of good quality. A blowing systolic murmur was localized at the mitral area. The abdomen was distended, dull to percussion and a definite fluid wave was elicited. A mass was palpable in the left upper quadrant, which was firm in consistence, irregular in outline and extended 3 cm. below the costal margin. The liver was palpated 3 cm. below the right costal margin. There was a reducible left indirect inguinal hernia containing a portion of intestine. The lower extremities were edematous and pitted on pressure. The skin was rough and dry and there was no abnormal pigmentation present. The hair was dry and lusterless and the nails and lips slightly cyanotic. The eyes reacted to light and accommodation. There was a moderate tortuosity of retinal vessels. The neurological examination revealed no noteworthy abnormalities. Rectal examination revealed a few hemorrhoidal tags and a spastic sphincter. The prostate gland was firm but not definitely enlarged.

*Clinical Laboratory Investigations:* The erythrocyte count on admission was 3,400,000 per cmm., and the total leukocyte count 10,700 per cmm. A differential count revealed 25 per cent neutrophils, 59 per cent lymphocytes and 16 per cent monocytes. The blood platelets were 496,400 per cmm. The erythrocytes comprised 39 per cent and the leukocytes 1 per cent of the packed cell volume. The actual sedimentation rate (Wintrobe) was 0.4 mm. per minute; the corrected sedimentation rate was 0.2 mm. per minute. Red cell fragility (Wiseman technic) ranged from 0.43 to 0.30. Urinalysis and renal function tests (concentration, dilution and phenolsulphonphthalein) were essentially normal. The blood urea nitrogen was 9.2 mg. per cent. A liver function test with bromsulphalein revealed a 90 per cent retention in 5 minutes

and less than 10 per cent retention in 30 minutes. The Wassermann and Kahn tests of the blood serum were positive (4 plus).

Complete hematological investigations were conducted daily. The total leukocyte count ranged from 10,700 per cmm. to 24,000 per cmm.; absolute counts ranged as follows: lymphocytes 6420 to 20,640 per cmm., monocytes 900 to 2288 per cmm., and granulocytes 1680 to 3900 per cmm. The erythrocyte count averaged 3,300,000 per cmm., and the hemoglobin varied from 10 to 12.8 gm. (Newcomer). The reticulocytes averaged 1.2 per cent. The blood platelets varied from 212,100 to 645,840 per cmm. The icterus index was 7, and the direct van den Bergh test was negative; the serum bilirubin was 0.3 mg. per cent.

*Clinical Course:* The patient's temperature fluctuated between 95.8° and 99° F. Five paracenteses were done for relief of abdominal distress, and approximately 2500 cc. of cloudy straw colored fluid were removed. Microscopic examination of the ascitic fluid by supravital technic revealed a preponderance of small lymphocytes, with large vacuolated clasmotocytic elements in every oil immersion field. There were 3 to 6 red blood cells per high power field, and an occasional polymorphonuclear leukocyte. In addition, an occasional large round cell with a large vesicular nucleus containing one or two prominent nucleoli, and a highly vacuolated cytoplasm containing refractile bodies was present. A biopsy of a small axillary lymph gland, measuring 1 cm. in diameter, revealed a moderate hyperplasia of lymphocytes without the characteristic appearance of lymphocytic leukemia.

After 30 days in the hospital the patient was discharged. Five days later he returned with marked distention of the abdomen, shortness of breath and urinary frequency. His temperature was normal and the pulse frequency was 100 per minute. About 4 liters of thin cream colored fluid were removed by abdominal paracentesis. Five days later about 8 liters of fluid of the same character were removed. On the 10th hospital day his pulse suddenly became weak and he became cyanotic and expired.

#### ABSTRACT OF AUTOPSY REPORT \*

The abdominal cavity contains a large amount (6000 cc.) of cream colored fluid of milky consistence. The peritoneum is granular and the omentum is adherent to the anterior abdominal wall. There are numerous adhesions between loops of the small intestines and the colon which are easily separated. There is a diffuse plastic exudate approximately 2 mm. in diameter covering the exposed peritoneal surfaces of the intestine and the mesentery.

The liver extends 5 cm. below the costal margin in the right mammary line. The edge of the liver is rounded and the surface is diffusely and finely granular. It weighs 2360 gm. and the cut surface is of a pale brown color and presents the fine lobulation seen in portal cirrhosis.

The spleen is 3 cm. below the costal margin and there are ad-

\* No. 436-999.

hesions between the omentum and the capsule of the spleen. The spleen weighs 1260 gm. and on cut section presents multiple infarctions. The capsule is slightly thickened and the pulp is moderately firm with irregular areas of hemorrhage.

*Lymph Nodes:* There is a marked enlargement of the lymph nodes at the root of the mesentery and those of the entire peripancreatic group. The nodes vary markedly in size from 1 to 4 cm. On cut section the glands bulge beyond their capsules. The cut surface presents numerous small cysts, from which may be expressed small fatty granules and a semisolid material. The cut surface presents a fairly uniform, creamy yellow appearance with occasional granules which are of a deeper yellow color. The larger nodes have entirely lost their normal appearance. The cut surfaces of the smaller nodes present small yellow granules embedded in the glandular tissue. The retroperitoneal group of lymph nodes (Fig. 1) extending from the coeliac axis to the brim of the pelvis are most markedly enlarged. Many of these nodes are the seat of recent hemorrhage. The mediastinal group of lymph nodes, although moderately enlarged and of similar appearance, are much smaller than the mesenteric and the retroperitoneal group. The superficial lymph nodes are grossly uninvolved by this process. The thoracic duct was not dissected out.

The mucosa of the jejunum and the ileum presents a diffuse, beefy red granular congestion without ulceration or gross deposits of material comparable to that present in the lymph nodes.

The kidneys weigh 410 gm. The surfaces are slightly granular and the cortex slightly thickened, measuring 1 cm. in diameter.

*Anatomical Diagnoses:* Chylous ascites, with plastic peritonitis; massive fat accumulations in mesenteric and retroperitoneal lymph nodes; portal cirrhosis, hepatomegaly; splenomegaly, with multiple infarcts; anemia; adenocarcinoma of prostate; nephrosclerosis; edema of lower extremities; left inguinal hernia; and atrophy of left testicle.

#### MICROSCOPIC EXAMINATION

*Small Intestines:* The villi are markedly broadened and blunted. The epithelium covering the villi is largely absent but well preserved when present. The outstanding characteristic of the villi is the marked dilatation of the lymphatics which with the hema-

toxylin-eosin stain presents the appearance of large empty spaces lined with endothelium. The stroma contains a large number of cells which are predominantly small lymphocytes. The serosa is covered with a fibrinous exudate in the meshes of which are numerous mononuclear cells which vary in appearance from the usual monocyte to large vacuolated macrophages of the foam cell type.

*Mesenteric Lymph Nodes:* The normal architecture of the lymph nodes is absent. The nodes contain numerous dilated spaces (Figs. 2 and 3) presenting the general aspects of lymph sinuses. These sinuses are more markedly dilated than in the cases of Jarcho and Whipple. They are filled with large, amorphous agglomerations, which when stained with sudan III, scharlach R and the Nile blue sulphate stain reveal the characteristic reactions for fat. Surrounding these fatty agglomerations are numerous vacuolated mononuclear macrophages and multinuclear giant cells (Fig. 2). Many of the spaces contain masses of these large foam cells without the fatty agglomerates (Fig. 3). The nuclei of the foam cells (Figs. 4-7) are frequently pyknotic, suggesting degenerative changes. The fat tissue surrounding the lymph nodes, blood vessels, pancreas and kidney contains numerous small lymphocytes presenting a leukemic type of infiltration. There are also focal aggregates of proliferating monocytic cells encountered in the kidney and in the mesenteric fat.

*Pancreas:* The parenchymatous cells and islands of Langerhans appear normal. There is a moderate dilatation of lymphatic vessels and pancreatic ducts, and focal areas of metaplasia of the epithelium of many of the small pancreatic ducts. There is a slight interstitial fibrosis of the pancreas and lymphocytic infiltration of the peripancreatic fat.

*Liver:* A moderate proliferation of bile ducts with portal fibrosis and a marked infiltration of lymphocytes is seen. There is no evidence of xanthomatous biliary cirrhosis.

*Spleen:* Marked passive congestion, hemorrhage, and numerous infarcts of varying ages are present. There is no evidence of nests of xanthomatous cells or deposits of fat with foam cells similar to those seen in the lymph nodes.

*Special Stains:* Numerous tissues were stained for *Treponema pallidum* but none was demonstrated. After comparison of sections

of lymph nodes stained for fat from this case with those from the cases of Whipple and Jarcho, Dr. VandeGrift noted that our case "contained a large amount of hyaline appearing material which takes a minimal fat stain, but is apparently lipid substance as there are small globules of deep staining fat in this material."

### DISCUSSION

The early clinical manifestations of this case were predominately those of a blood dyscrasia, which was carefully studied in its hematological aspects. These studies led to a tentative diagnosis of a benign pseudoleukemic lymphocytosis, relative neutropenia, and a moderate hypochromic microcytic anemia. With the development of chylous ascites an obstruction of the thoracic duct due to lymphadenosis or carcinoma was postulated. Unfortunately the thoracic duct was not dissected out at autopsy. However, in the case reported by Whipple, and the 2nd case of Blumgart's series, the thoracic duct was dissected out and no anatomical obstruction was demonstrated.

In contrast with the cases reported by Whipple, Blumgart, and Jarcho, fatty diarrhea was not a prominent clinical symptom in our case and there was no evidence of rheumatic fever.

The massive deposition of fat in the sinuses of the mesenteric and retroperitoneal lymph nodes represents a quantitative increase of lipids. While obstruction of the thoracic duct may constitute the basis of such a condition, gross anatomical evidence of such obstruction is not available. Cases in which anatomical obstruction of the thoracic duct have been demonstrated have presented the clinical manifestations of steatorrhea without anatomical evidence of depositions of fat in the lymph nodes.<sup>5</sup>

Dilated lymphatics and lymph sinuses with massive deposits of fat, largely confined to the mesenteric and retroperitoneal lymph nodes, would scarcely be expected to originate from inadequate intestinal absorption, but malabsorption may later supervene. Although primary lymphatic obstruction cannot be eliminated, an analogous functional result may follow increased intestinal absorption of fat. This predicates increased amounts of fat in the intestines from food intake, intestinal excretion of fat, or impaired intestinal cholesterol destruction. Recent studies of lipid metabolism suggest that the "enormous increase in the amount of fat in

the feces, as in obstructive jaundice, may be due largely to increased excretion of fat rather than to diminished absorption, as was formerly believed to be the case." <sup>6</sup> Emaciation and depletion of body fat, one of the outstanding clinical manifestations of these cases, suggest the possibility of increased excretion of fat into the intestines. Inadequate data are available to suggest a change of the bacterial flora of the intestines which might increase cholesterol absorption. The evidence suggests massive excretion of fat into the intestines and an increased reabsorption of fat from the intestines. The term "intestinal lipodystrophy" as suggested by Whipple <sup>1</sup> would seem to be most appropriate.

The relation of this condition to the xanthomatous diseases seems less marked. Thannhauser and Magendantz <sup>7</sup> in their investigations of the xanthomatous diseases suggest that the basic disturbance lies in the xanthoma cell rather than in intermediary cholesterol metabolism. Thus, the xanthomatous nodules are not produced by a deposit of cholesterol outside the cells, but by a disease of certain cells which contain cholesterol intracellularly. In contrast with the xanthomatous diseases these cases present large aggregates of extracellular as well as intracellular fat. Furthermore, a fundamentally different type of cell is probably involved (Figs. 4-7).

#### SUMMARY

A case of intestinal lipodystrophy analogous to that first described by Whipple is reported. Five similar cases have been previously reported.

Intestinal lipodystrophy is characterized by progressive emaciation, chylous ascites, fatty diarrhea, mild hypochromic microcytic anemia, a characteristic anatomical pattern and a fatal termination.

The anatomical pattern consists of the presence of large aggregations of hyaline and fatty material surrounded by large foam cells in dilated sinuses of the mesenteric and retroperitoneal lymph nodes, and the mucosa of the small intestines. These lymph nodes are markedly enlarged, and the lymphoid tissue is largely replaced as a result of this process.

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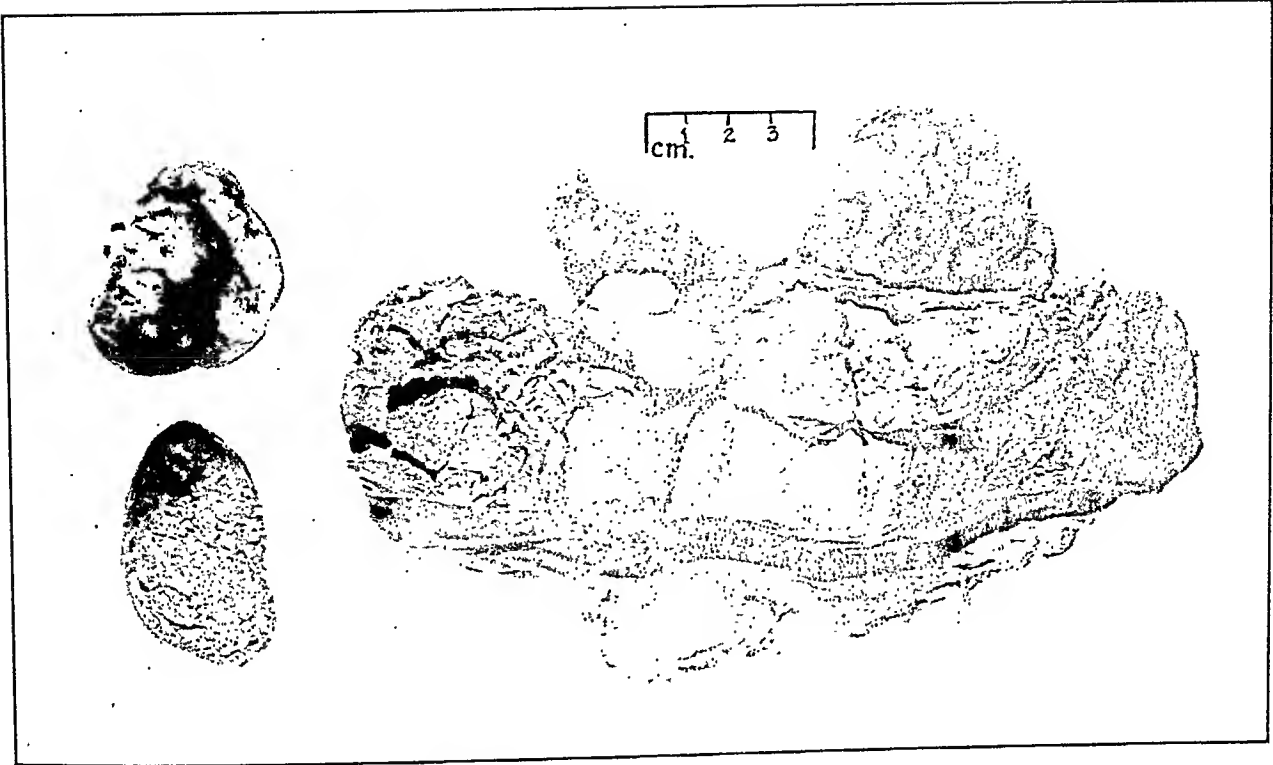


## DESCRIPTION OF PLATES

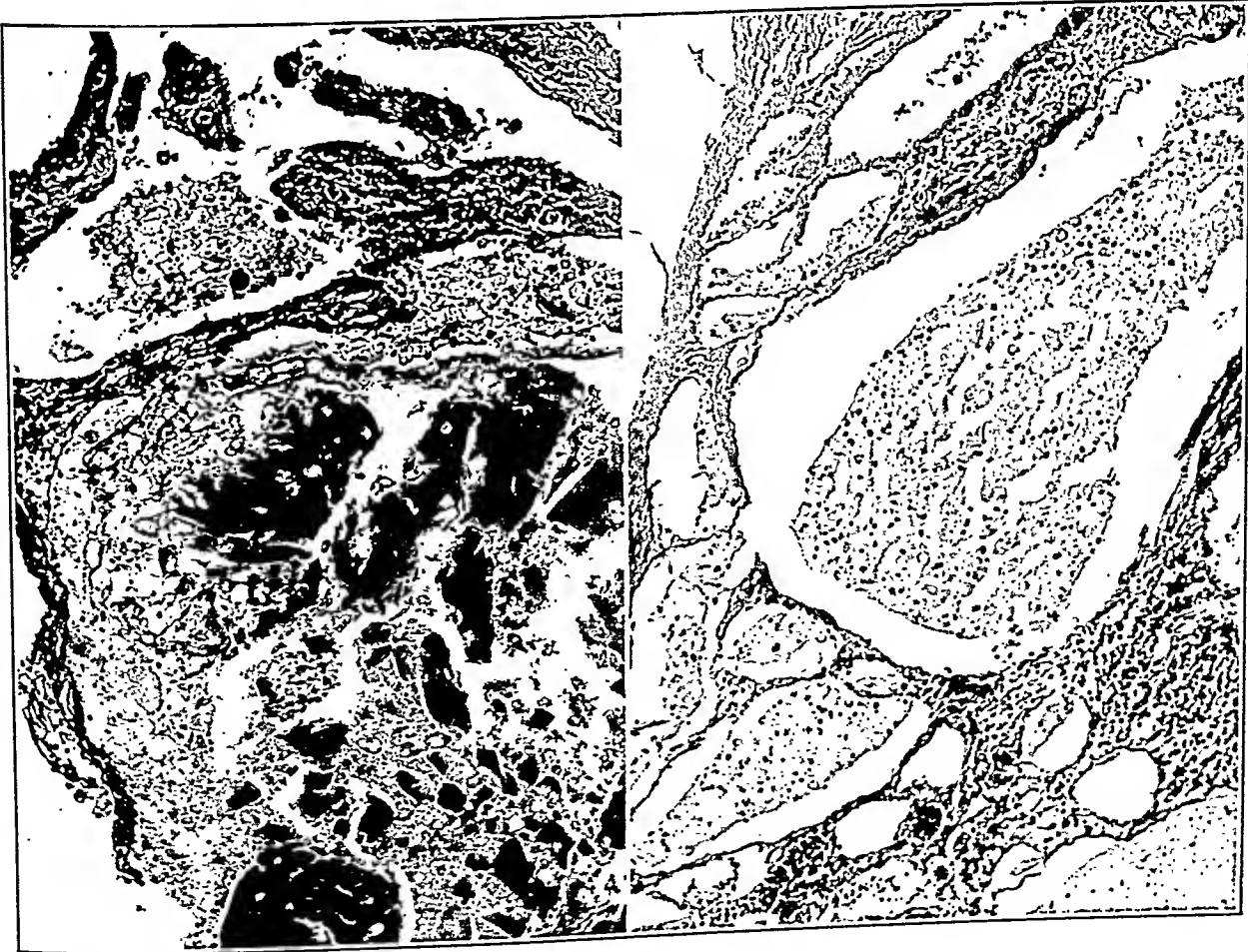
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### PLATE 80

- FIG. 1. A large group of retroperitoneal lymph nodes containing yellow granules. Hemorrhagic areas are present in some of the nodes.
- FIG. 2. Section from a retroperitoneal lymph node showing large fatty agglomerates margined by mononuclear foam cells and multinucleated giant cells.
- FIG. 3. Section from a peritoneal lymph node showing markedly dilated lymph sinuses containing large mononuclear phagocytic foam cells and multinucleated giant cells.



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Malabsorption of Fat

PLATE 81

FIGS. 4, 6 and 7. Mononuclear phagocytic cells with vacuolated cytoplasm and foam cells.

FIG. 5. Large multinucleated giant cells with vacuolated cytoplasm.



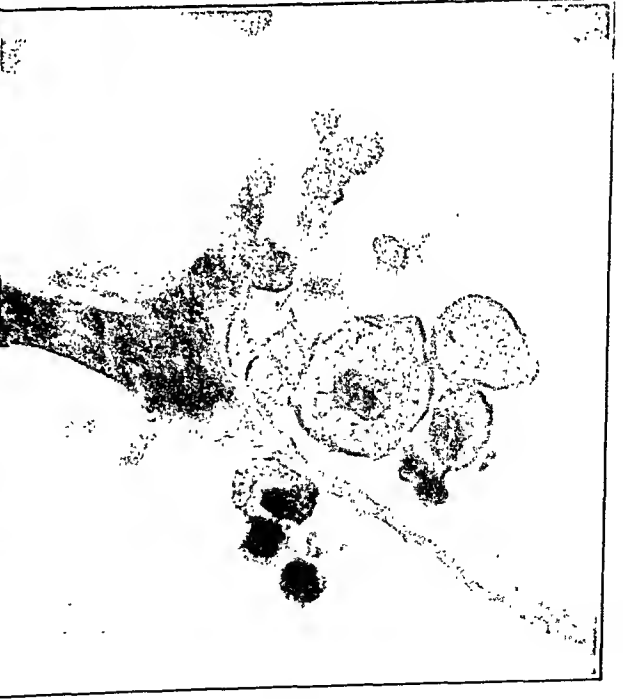
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# THE EFFECT OF CERTAIN FACTORS ON THE RESULTS OF SILVER IMPREGNATION FOR RETICULUM FIBERS \*

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This paper deals with the results of further experiments carried out in order to determine the effect of various factors on the results of silver impregnation of reticulum. The results of the first set of experiments were published in 1937,<sup>1</sup> together with the description of a simple method for the impregnation of reticulum in paraffin sections.

*The Effect of Different Fixatives:* As the results of most silver impregnation methods, either for reticulum or for other tissue elements, are known to depend on the type of fixation, and as in most instances the use of some specified fixative such as formalin, cobalt nitrate-formalin, alcohol, chloral hydrate, and others is recommended, it seemed to be of interest to ascertain the actual effect of different fixatives on the results of the stain. The following fixatives were used: alcohol, Carnoy's fluid, formalin-alcohol (1:5), formalin (1:10), Bouin's, Orth's, Zenker's and Stieve's fluids, and neutral Zenker-formalin (9:1). The material used was various human and animal (dog, guinea pig) organs, mainly kidneys, liver, spleen, heart muscle and pancreas. The impregnation of paraffin sections was done according to the method previously published.

It should be stated first of all that for practical purposes any one of the fixatives tried gives entirely satisfactory results. The reticulum fibers show up quite distinctly and selectively, and in about equal numbers after any of the fixatives. The difference is to be found in the staining of the nuclei and of the cytoplasm much more than in the reticulum stain itself. The best results consisting of deep black staining reticulum fibers, sharply stained gray nuclei and almost completely unstained cytoplasm, are obtained after fixation in Carnoy's fluid. A somewhat darker and often purplish cytoplasmic stain with a varying nuclear stain is

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obtained after Zenker's fixative. The results obtained with Zenker-formalin are quite similar. After fixation in Bouin's fluid the nuclei are entirely unstained while the cytoplasm is often darker than desirable. With all the other fixatives the results do not differ materially from those obtainable after formalin fixation. There can be no doubt that from an aesthetic point of view the results after fixation in Carnoy's fluid are much superior to those secured with other fixatives. I have found, however, that results almost identical with those obtained by fixation in Carnoy's fluid can be obtained after any fixative with a slight modification of the technic which I call "exhaustive oxidation" of the sections. This term means thorough oxidation of all tissue substances oxidizable with potassium permanganate. The potassium permanganate solution should be acidified with about 0.5 per cent sulphuric acid, and after decolorization with potassium metabisulphite the process should be repeated as often as there is the slightest brown staining of the sections by potassium permanganate (as a rule 2 to 3 times). Pale pink staining of the sections indicates the complete exhaustion of all substances capable of reducing potassium permanganate. After this type of oxidation subsequent mordanting and silver impregnation will bring out surprisingly sharp black and white contrasts. Sections must be affixed to the slide securely because they are easily loosened by oxidation and have a great tendency to float off.

*Length of Time of Fixation:* This does not seem to make much difference, at least not with formalin fixation, as tissues fixed for from 12 hours up to several months gave almost identical results.

*Decalcification:* Decalcification in either nitric or sulphosalicylic acid does not call for any modification in the technic as it has no effect whatsoever on the results of the impregnation.

*Thickness of Sections:* The thinner the sections, the better the result. With thick sections the picture is often blurred, the background being stained an unpleasant greenish or brownish gray, while the reticulum fibers are exceedingly pale. The limit of safety is about  $8\mu$ , though occasionally excellent results may be obtained with sections as thick as  $16\mu$ . Celloidin sections, either stained loose or affixed to slides, often show the same poor staining, especially if the celloidin has not been removed. As the importance of diffusion processes in silver impregnation is well known (an

especially interesting example being given in the Warthin and Starry technic<sup>2</sup>), it is by no means surprising that the thickness of the sections may not only quantitatively but also qualitatively influence the results.

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## A DIFFERENTIAL STAIN FOR CELL TYPES IN THE PANCREATIC ISLETS \*

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Most, if not all, of the accepted staining methods for the differentiation of cell types in the islets of Langerhans are more or less capricious and unreliable. The neutral gentian violet stain gives beautiful results in guinea pig material, but it is much less dependable in other animal species. The Mallory-Heidenhain azan method can be applied to almost all animal species, but gives a clear definition of the *alpha* granules only, while the *beta* granules are poorly demonstrated. The other methods (copper and iron hematoxylin, acid fuchsin-methyl green) are even less satisfactory than the stains mentioned.

The routine hematoxylin-eosin stain shows in well fixed tissues a definite difference between *alpha* and *beta* cells owing to the relative oxyphilia of the former and the basophilia of the latter. As a result of my attempts to increase this contrast I have found a fairly simple modification of the hematoxylin-eosin stain which brings out the two cell types more distinctly than any of the other stains used for the same purpose. It has the additional advantage of giving uniform results in all animal species examined (guinea pig, rat, mouse, cat, dog, rabbit, *Macacus rhesus* monkey, beef) as well as on human material. Its results were excellent in certain human cases in which all other stains failed. The two cell types were clearly demonstrated in both the normal and the diabetic pancreas sometimes taken as late as 4 hours after death. This method is also suitable for study of the degranulation processes in the pancreas in experimental animals. It can also be recommended for the pituitary. In the pancreas the *beta* granules stain a deep slate blue color, while the *alpha* granules stain red. The D cells of Bloom are not demonstrated. In the pituitary the basophils are blue, the oxyphils red, and the chromophobes are almost unstained.

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## METHOD

*Fixation:* The thickness of pieces of tissue should not exceed 2 mm. in order to ensure rapid and complete penetration by the fixative. Aqueous fixatives, such as formalin, Bouin's, Zenker's, and Stieve's solution, or Zenker-formalin, may be used. The best fixative proved to be a modified Bouin's solution in which half the amount of acetic acid is replaced by sulphosalicylic acid (formalin 1 part, saturated picric acid solution 4 to 5 parts, acetic and sulphosalicylic acid 2.5 per cent each). For use dilute this fixative with equal parts of distilled water. The staining of tissues fixed in any fluid can sometimes be greatly improved by refixing the sections before staining in the undiluted solution for 12–24 hours. The removal of mercury salts from tissues is done by the routine iodine method.

*Embedding:* Embed tissues in paraffin.

*Oxidation:* Deparaffinized (and refixed, if necessary) sections are treated for 1 minute with a solution containing about 0.3 per cent each of potassium permanganate and of sulphuric acid. The sections are rinsed in water and then decolorized with a 1 to 5 per cent solution of potassium metabisulphite. After decolorization the sections are thoroughly washed in water. Without oxidation the *beta* granules will not take the stain. For sections of pituitary, oxidation, though it will enhance the color contrast, is not strictly necessary.

*Staining:* Stain in a well ripened solution of chromium hematoxylin for from 15 minutes to 1 hour under microscopic control. The *beta* granules should be a deep blue and the cytoplasm of the *alpha* cells should be unstained. In the pituitary the basophils should be a deep blue and the oxyphils unstained. Any mucoid material is stained a deep blue. The staining solution is made up as follows:

Mix equal parts of a 1 per cent aqueous solution of hematoxylin and of a 5 per cent solution of chromium alum (potassium chromium sulphate). The brownish mixture is ripened by the addition of about 3.5 cc. of a 5 per cent potassium dichromate solution plus a few drops of sulphuric acid to each 100 cc. of the mixture. The process of ripening will take 1 or 2 days. The solution is ready for use as soon as it is a deep blue-black with a

purplish tinge. It should be filtered before use as a precipitate will form repeatedly on its surface. For some time the staining power and selectivity of the solution increase, but as the solution becomes older the staining time has to be prolonged. Solutions that do not stain deeply enough in 35 to 40 minutes are better discarded as they often do not stain with sufficient precision.

After staining in this chromium hematoxylin solution rinse the sections in water and transfer to alcohol containing 1 per cent of hydrochloric acid. In this acid alcohol solution the color of the section becomes a clearer blue. Rinse again and counterstain rather heavily with any of the routine red or orange acid dyes. Phloxine and ponceau de xylinine have been found to be especially suitable. The latter is used in a 0.5 per cent solution with 1 per cent of acetic acid. Rinse in water and differentiate in a 5 per cent solution of phosphotungstic acid until the acid dye is completely removed from the connective tissue. Only the strongly oxyphilic structures, such as erythrocytes, muscle fibers, oxyphils and *alpha* cells, remain stained. Carry sections through graded alcohols, clear in xylol and mount in balsam.

## DESCRIPTION OF PLATE

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### PLATE 82

Tissues were fixed in modified Bouin's solution and stained according to the method recommended. Phloxine was used as a counterstain.

An orange-yellow filter was used for photographing the sections. The blue stained tissue elements are dark and the pink stained elements pale. All microphotographs were taken at a magnification of  $\times 380$ .

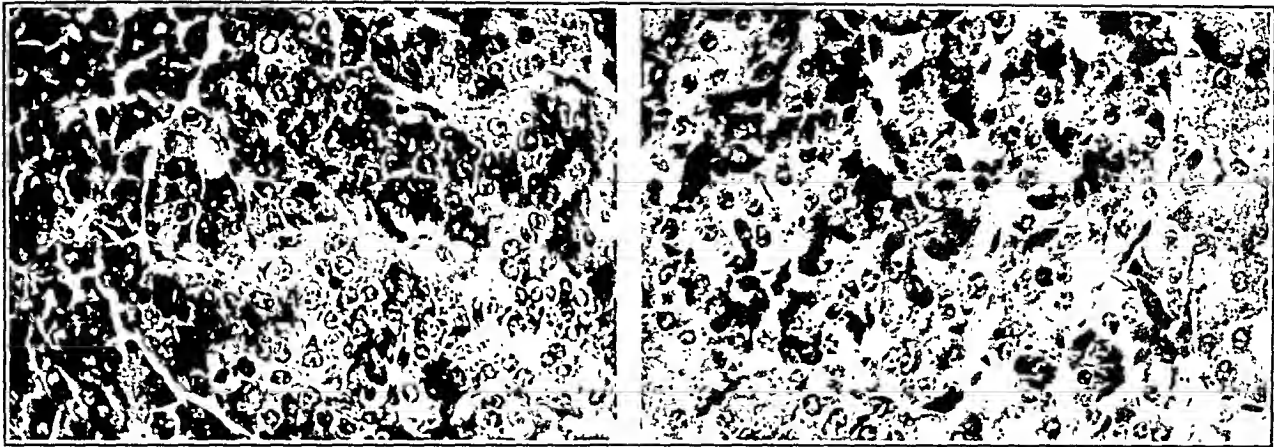
FIG. 1. Pancreas from a normal guinea pig. A = pale *alpha* cells; B = dark *beta* cells.

FIG. 2. An islet in the pancreas of a guinea pig. The blood sugar level was kept at 200 mg. per 100 cc. for 12 hours preceding death. Profound degeneration of the *beta* cells, of which only a few show very deep blue granules in a cap-shaped area of the cytoplasm, is to be seen.

FIG. 3. Normal human islet. A = pale *alpha* cells; B = dark *beta* cells.

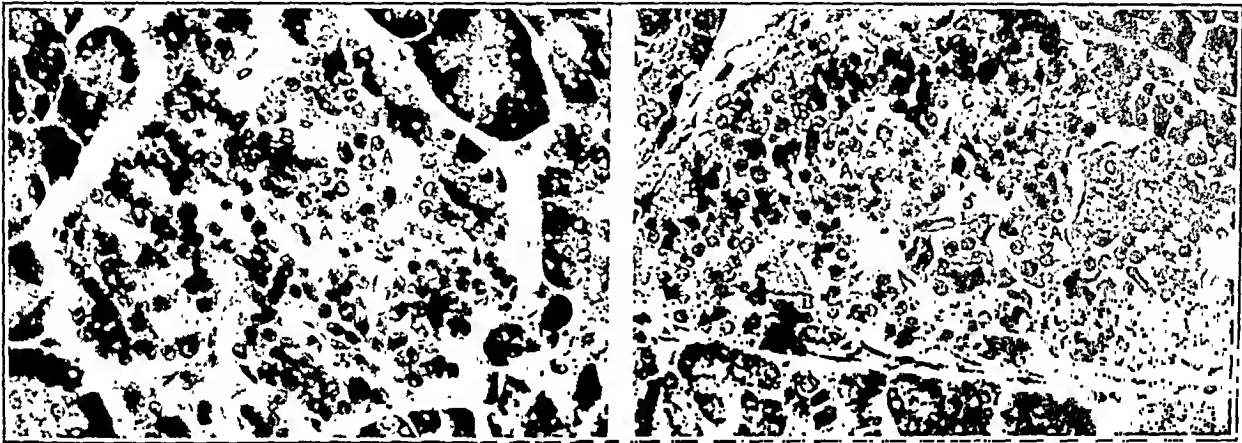
FIG. 4. Human pancreas from a case of severe diabetes. A = pale *alpha* cells; B = dark *beta* cells; C = masses of hyalin.

FIG. 5. Anterior lobe of a human pituitary gland. A = chromophobes with unstained cytoplasm; B = dark basophils; C = pale oxyphils.



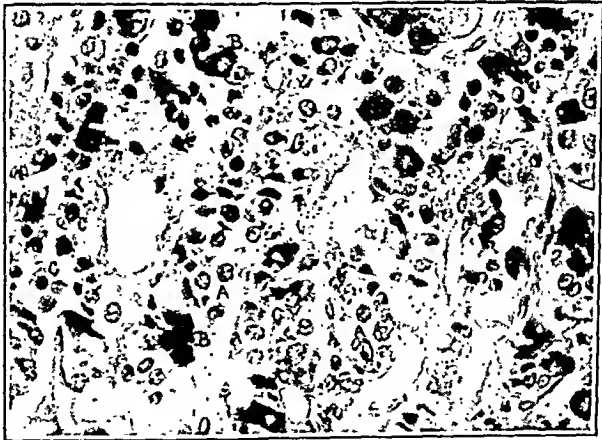
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Gomori

Stain for Cell Types in Pancreatic Islets



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## THE OCCURRENCE OF VIRULENT TUBERCLE BACILLI IN PRESUMABLY NON-TUBERCULOUS LUNG TISSUE \*

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Section on Pathological Anatomy, The Mayo Clinic, Rochester, Minn.)*

In 1927 Opie and Aronson<sup>1</sup> reported the results of a comprehensive study to determine whether or not latent focal lesions of pulmonary tuberculosis contain virulent tubercle bacilli. A total of 304 lesions obtained from the lungs of 169 individuals dying of causes other than tuberculosis in two Philadelphia hospitals was examined. Data pertaining to 208 lesions of the primary complex from the lungs and tracheobronchial lymph nodes, which were used to inoculate guinea pigs, revealed that tubercle bacilli were present in approximately 26 per cent. Since the material examined by Opie and Aronson consisted of focal lesions that were caseous, caseocalcareous, calcified or encapsulated, the authors questioned whether the tubercle bacilli, in the instances in which the results of the inoculation of guinea pigs were positive, were present within the structure of the focal lesions or in the peripheral tissue in which the lesions were embedded. They accordingly investigated this phase of the problem, injecting guinea pigs with (1) lung tissue from the apexes, which were grossly without evidence of tuberculosis or fibrous scars; (2) lung tissue presumably free from tuberculous lesions, from the base of the lungs; and (3) apparently non-tuberculous lymph nodes from the hilum or the tracheobronchial tree. It was found that tubercle bacilli were present in tissues removed from 15, or 45.5 per cent,

\* Received for publication April 29, 1939.



of the 33 individuals in whom the character of latent tuberculosis consisted of fibrocaseous lesions of the apexes, fibrous scars of the apexes and caseous encapsulated or calcified nodules of the lungs and contiguous lymph nodes.

In a previous communication<sup>2</sup> we reported the results of a study in which attempts were made by cultural procedures and by the inoculation of guinea pigs to demonstrate tubercle bacilli in chronic tuberculous lesions of the lungs and contiguous lymph nodes of individuals dying of causes other than tuberculosis. Material from a total of 68 cases was utilized and tubercle bacilli were demonstrated in only 1 instance. In the study just referred to an attempt was made in so far as was practical to use for subsequent study only those tissues that constituted the lesions of tuberculosis and to discard as much as possible of the surrounding tissues which, while apparently non-tuberculous, were found in Opie and Aronson's<sup>1</sup> series to contain virulent tubercle bacilli in a high percentage of cases. The occurrence in this study of only 1 positive result in material from 68 autopsies suggested the desirability of an additional study in which would be investigated the possibility of virulent tubercle bacilli occurring, as Opie and Aronson had found, in the presumably non-tuberculous tissues of the lung and contiguous lymph nodes.

#### MATERIAL AND METHODS

Only material from unembalmed bodies was selected. The specimens for subsequent study were obtained at the time of autopsy and a separate set of sterile scissors and forceps was used for the removal of each specimen. Each specimen was placed in a sterile container for transmission to the laboratory.

With one exception only bodies of persons dying of causes other than tuberculosis were included in the study. With the one exception noted, the individuals from whom the material was obtained showed no clinical signs of tuberculosis or anatomical evidence of active tuberculosis at the time of autopsy.

The material was obtained at autopsy from 51 individuals. In all but 12, lesions of so-called latent or chronic tuberculosis were noted at the time of the postmortem examination. In most instances the signs of tuberculosis consisted grossly of non-active or presumably healed lesions usually referred to as the Ghon or

primary complex. A summary of lesions that were designated as tuberculous in character is as follows: healed Ghon complex of the lungs with no lesions of tuberculosis elsewhere, 21 cases; healed Ghon complex with healed lesions in the liver alone or in the liver and spleen in combination, 5 cases; Ghon tubercle of lung only, 1 case; healed lesions of the hilar lymph nodes only, 8 cases; healed lesions of the hilar lymph nodes and liver, 2 cases; and healed tuberculosis of the apical lobe, 1 case. (In 1 case the patient had tuberculous apical scars of the lungs and died of a tuberculous enteritis and peritonitis.) In 25 of the cases the lesions of tuberculosis were associated with so-called "apical scars."

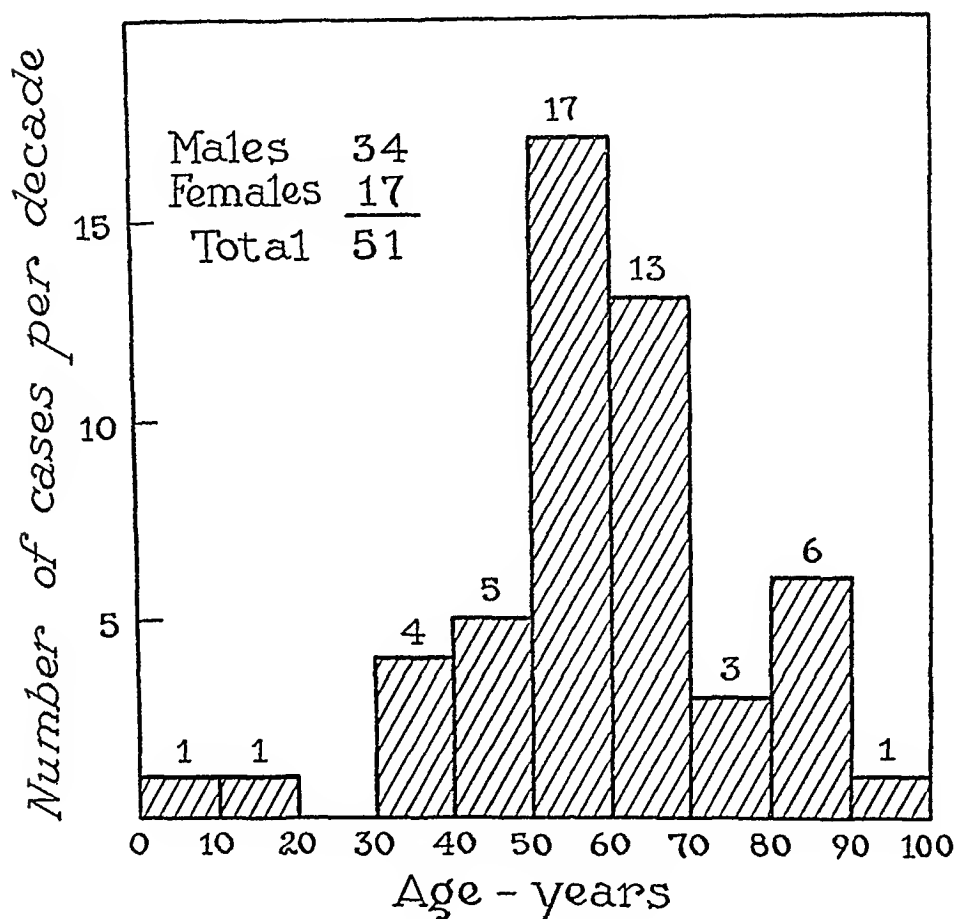
The ages of the respective individuals comprising the 51 cases ranged from 2½ to 93 years with the largest number between 50 and 69 years inclusive (Text-Fig. 1). All were white. Thirty-four were males and 17 were females. The environment of 13 was rural and of 38 urban.

In securing material to be examined for the presence of tubercle bacilli only tissue that appeared grossly to be non-tuberculous was selected. If focal lesions of presumably healed tuberculosis were present, care was taken to select the specimen at some distance from the focal lesions rather than immediately adjacent to them. Although the procedure followed varied slightly in a few instances, the material obtained from each body consisted of portions of both upper and both lower lobes of the lungs and the hilar or tracheobronchial lymph nodes.\* A composite emulsion was prepared from both of the upper lobes and the same procedure was followed for both of the lower lobes. Hilar or tracheobronchial lymph nodes were prepared as one emulsion. In most instances, therefore, there was available from each case 3 emulsions for inoculation into guinea pigs.† The entire liquid portions of the respective emulsions were divided between 2 guinea pigs, the injections being made subcutaneously. The guinea pigs were kept un-

\* An idea of the amount of lung tissue secured for study may be obtained from the following. In 25 of the cases the average weight of the tissue from both upper lobes was 11.2 gm. and the average weight of the tissue from both lower lobes was 14.5 gm.

† Cultures were also attempted from the emulsions prepared from the first 25 cases, but since the results were all negative it was thought advisable to utilize all of the subsequent material for the injection of guinea pigs.

der observation for 8 weeks, after which time they were killed and examined for evidence of infection with tubercle bacilli. At the time of autopsy the guinea pigs were examined carefully and any abnormality that appeared to resemble tuberculosis even remotely was submitted to subsequent study, which included his-



TEXT-FIG. 1. Age distribution by decades of the 51 individuals from whom material was obtained for study.

tological examination, cultural attempts to demonstrate tubercle bacilli, and in some instances the inoculation of additional guinea pigs with an emulsion of the tissue in question.

A summary of the results of the animal inoculation tests shows that from the 51 cases there was prepared for the inoculation of guinea pigs a total of 150 emulsions. These consisted of 51 composite emulsions prepared from portions of both upper lobes, 51 composite emulsions prepared from portions of both lower

lobes, and 48 composite emulsions of hilar and tracheobronchial lymph nodes. A total of 300 guinea pigs was inoculated and 265 of these lived 3 weeks or longer after being inoculated. In fact, death among the inoculated guinea pigs from intercurrent infections seldom occurred after the first 3 weeks. Although 35 guinea pigs were recorded as "failures," since they died within 3 weeks after being inoculated, the majority of the animals were living at the end of 8 weeks, when they were killed. In only 6 instances did both guinea pigs that were inoculated with the same emulsion die during the first 21 days. In no instance did all of the guinea pigs in a group inoculated with material from the same case die before the period usually necessary for the recognition of the lesions of experimental tuberculosis.

### RESULTS

From the results of the autopsy findings in the guinea pigs, tubercle bacilli were demonstrated in only 3 of the 51 cases (Table I). The salient features concerning the clinical history and the pathological findings in these cases follow.

CASE 1: This patient, a white woman of 78 years, had been confined in a psychopathic hospital for 25 years because of a manic depressive psychosis. In 1936 her neck had become swollen and inflamed and persistent draining sinuses had developed. In July, 1937, she suffered a fracture of the neck of the right femur. Following this she failed gradually. Two days before death she had numerous tarry stools.

At autopsy tuberculous enteritis and peritonitis with a severe gastro-intestinal hemorrhage were found. There was a tuberculoma between the stomach and the pancreas, and tuberculous nodules were found in the liver and in the lymph nodes of the right supraclavicular region. The cervical lymph nodes were not examined.

The only gross evidence of tuberculosis of the lungs was bilateral apical scarring graded 3 on a basis of 1 to 4. The scar at the right apex was calcified.

*Histological Examination:* The section of the decalcified right apical scar revealed a small mass of hyalinized connective tissue partially surrounded by a small group of lymphocytes. There were many dilated bronchioles embedded in a basophilic connective tissue. Groups of lymphocytes were numerous around the bronchioles but nowhere was there evidence of active tuberculosis. The

section of the left apical scar had a similar appearance. None of the sections from the lung or hilar nodes taken from regions contiguous to those from which specimens were taken for inoculation revealed any evidence of tuberculosis either active or healed.

TABLE I

*Summary of the 3 Cases from which Virulent Tubercle Bacilli Were Demonstrated*

Case	Age	Sex	Tuberculosis at autopsy	Inoculum *	Results
1	yrs. 78	Female	Bilateral apical scars. Miliary tubercles in liver and spleen. Tuberculous enteritis and peritonitis	Upper lobes of both lungs exclusive of apical scars	Both guinea pigs tuberculous
				Lower lobes of both lungs	Both guinea pigs negative
				Hilar lymph nodes	Both guinea pigs negative
2	65	Female	Healed primary complex of lungs. Bilateral apical scars	Upper and lower lobes of right lung	Both guinea pigs negative
				Upper and lower lobes of left lung	One guinea pig negative. Second guinea pig tuberculous and culture obtained from spleen
				Hilar lymph node	Both guinea pigs negative
3	85	Male	Healed primary complex of lungs. Bilateral apical scars	Upper lobes of both lungs	One guinea pig negative. Second guinea pig died after 2 days
				Lower lobes of both lungs	Both guinea pigs negative
				Hilar lymph nodes	One guinea pig negative. Second guinea pig tuberculosis limited to spleen

\* Each inoculum prepared from the lungs represented approximately 10 to 14 gm. of tissue.

Sections of the organs grossly involved by tuberculosis revealed evidence of an active tuberculous process attended with much caseation. In addition to the organs involved grossly, histological examination of the spleen revealed occasional tubercles.

*Animal Inoculations:* Material for the inoculation of guinea pigs consisted of tissue from the apparently normal part of the upper lobes of both lungs, the same from both lower lobes, and lymphoid tissue from the region of the hilum. Six guinea pigs were injected and in only 2 were lesions of tuberculosis found when they were killed 8 weeks later. Both of the animals in which lesions of tuberculosis were found had received portions of the composite emulsion prepared from the upper lobes of the lungs.

The tuberculosis present in 1 of the guinea pigs was minimal in amount, while in the other animal there was extensive involvement of the spleen and a few tuberculous foci in the lungs. From the spleen of this animal a culture with the physical characteristics of the human form of the tubercle bacillus was obtained.

CASE 2: This patient, a white woman of 65 years, had previously been a resident of a city of 30,000 population. There was no history of tuberculosis in the immediate family. She had had convulsions about once a month since the age of 34 years. She was poorly nourished and disoriented. Nine days before her death she was committed to a psychopathic hospital. She failed gradually and died after repeated convulsions.

At autopsy it was found that she had an ancient cerebral infarction in the right occipital region with a dilatation of the posterior horn of the left lateral ventricle. She was emaciated grade 3 +. The only anatomical evidence of tuberculosis was the presence of bilateral apical scars graded 1 + and an apparently healed Ghon complex. The Ghon tubercle was located in the lower lobe of the right lung and the lesion in the hilar node was small and calcified. These lesions were not found grossly until after they had been located by roentgenographic studies of the lungs removed at autopsy.

*Histological Examination:* Sections taken from regions contiguous to the specimens removed for inoculation experiments showed no evidence of tuberculosis, either active or healed. No sections were taken of the Ghon tubercle or the corresponding lesion in the hilar node.

*Animal Inoculations:* This was one of the few instances in which material from the upper and lower lobes of the same lung was used to inject guinea pigs. The usual procedure was to prepare a composite emulsion from tissue from both upper lobes and a second emulsion using tissue from both lower lobes. Six guinea

pigs were injected as follows: 2 with the combined material from the upper and lower lobes of the right lung, 2 with the combined material from the upper and lower lobes of the left lung, and 2 with lymphoid tissue from the region of the hilum. All of the guinea pigs lived for the required observation period of 8 weeks. At autopsy all of the guinea pigs were free of demonstrable lesions of tuberculosis except 1. This animal had received an inoculum prepared from the upper and lower lobes of the left lung. The tuberculous lesions in this guinea pig consisted of a large number of tuberculous nodules in the spleen, a few small foci in the liver, and a moderate number of tuberculous foci in the lungs. The tuberculous character of the lesions was confirmed by a microscopic examination of the respective organs and by the isolation from the spleen of virulent acid-fast bacilli.

The character of the culture obtained was dissimilar to that of the usual strains of tubercle bacilli of the human type and was subjected to further study. Although material from the tuberculous spleen of the guinea pig was used to inoculate both glycerinated and non-glycerinated mediums, growth occurred only on the mediums that contained no glycerin.\* Of the four tubes of non-glycerinated medium inoculated with material from the spleen of the guinea pig, growth was noted 73 days later in 3. The growth consisted of a few small discrete colonies in two tubes and innumerable small colonies on another. The colonies were non-chromogenic and even inclined to be colorless and had a luster. Miscibility in a saline solution was poor. The non-glycero-philic character of the culture was maintained in subsequent transfers.

The failure of the culture to grow in the presence of glycerin, which substance appears to enhance the growth-producing propensities of most culture mediums for the human form of the tubercle bacillus, provided sufficient reason to determine the pathogenicity of the culture for rabbits. Consequently 2 rabbits and 2 additional guinea pigs were each inoculated with 0.01 mg. of the culture obtained from the spleen of the guinea pigs previously mentioned.†

The results of these tests of pathogenicity indicated quite defi-

\* The medium used was the egg yolk-agar combination described originally by Capaldi<sup>3</sup> and more recently by Herrold.<sup>4</sup>

† The guinea pigs were injected subcutaneously, the rabbits intravenously.

nately that the infective organism was a tubercle bacillus of the bovine type.\* One of the rabbits died after 20 days and the other after 22 days. The lesions in the 2 animals were essentially alike. In both animals the spleen was swollen — in 1 tremendously so — and the lungs were literally filled with innumerable miliary foci indicative of aggravated and progressive tuberculosis.

CASE 3: This patient, a white man of 85 years, had lived in a city of 400,000 population and had been confined to a psychopathic hospital 3 months previous to his death because of a senile psychosis. There was no history of tuberculosis in his family. He remained bedfast and developed bronchopneumonia, from which he died. At autopsy he was found to have generalized and cerebral arteriosclerosis with multiple infarcts of the brain. There was bilateral bronchopneumonia and grade 3 + coronary sclerosis with chronic infarction of the interventricular septum of the heart.

The only gross evidence of tuberculosis of the lung was bilateral apical scarring, grade 1 +, a very small calcified lesion in a hilar node of the right lung and a hyalinized tubercle measuring 2 mm. in diameter in the liver.

*Histological Examination:* Sections of the apical scars revealed no evidence of tuberculosis. Sections taken from regions in the lungs and hilar nodes contiguous to those from which tissue was removed for the inoculation experiments revealed no evidence of tuberculosis. No sections were taken of the lesions of the hilar node or the liver.

*Animal Inoculations:* Two guinea pigs were inoculated with material from the combined specimens of tissue from the upper lobe of each lung, 2 with the material prepared from the combined specimens of tissue from the lower lobe of each lung, and 2 with an emulsion prepared from the lymphoid tissue from the region of the hilum. One of the guinea pigs inoculated with material from the upper lobes died 2 days later. The other 5 guinea pigs were living at the end of 8 weeks when they were killed for autopsy. In only 1 of them was evidence of tuberculosis found. This animal was 1 of the 2 that had been inoculated with material prepared from the lymphoid tissue from the hilum. Grossly the spleen showed four or five nodular lesions, apparently composed of conglomerate tubercles. The liver and lungs were not involved,

\* In order to verify this conclusion the organism was "typed" a second time using guinea pigs, rabbits and chickens. The results indicated that the organism was not pathogenic for chickens but was markedly so for guinea pigs and rabbits.



either grossly or microscopically. Microscopically the character of the changes in the spleen of the guinea pig was that of a mild rather than a severe tuberculous infection. There occurred diffuse nodules of epithelioid cells and histiocytes with a minimal amount of necrosis. A considerable portion of the spleen was apparently normal. In appropriately stained sections a few typical acid-fast bacillary forms were noted.

An attempt was made to secure cultures of tubercle bacilli from the spleen but it was unsuccessful. A portion of the splenic emulsion was used to inoculate 2 additional guinea pigs, 1 of which died 38 days and the other died 54 days after inoculation. Both animals showed a limited tuberculosis of the spleen and in 1 the liver was also slightly involved. Cultures were attempted from the spleen of the animal that died after 38 days and many colonies appeared on both glycerinated and non-glycerinated mediums. The characteristics of the cultures were those of the human tubercle bacillus.

#### COMMENT

In addition to the report of Opie and Aronson,<sup>1</sup> previously mentioned, there are relatively few reports of investigations dealing with the latent phase of tuberculous infections. This is especially true as regards the question of latency of pulmonary infection, in which search for tubercle bacilli has been made of the presumably non-tuberculous parenchyma of the lung.<sup>5</sup>

In 1890 Loomis<sup>6</sup> examined by the intrapleural inoculation of rabbits the apparently normal bronchial lymph nodes of 30 adults and obtained positive results in 8. Pizzini<sup>7</sup> in 1892 demonstrated by guinea pig inoculations the presence of tubercle bacilli in 12 of 30 adults in whom visible lesions of tuberculosis were not evident. Ten of the 12 positive results were obtained from bronchial lymph nodes and 2 from cervical lymph nodes. In these latter 2 the bronchial nodes also gave positive results.

In a report by Spengler<sup>8</sup> in 1893 an account was given of the finding by histological methods of tubercle bacilli in the bronchial lymph nodes of 6 children who had died of acute febrile diseases. Other evidence of tuberculous infection was not observed. Straus<sup>9</sup> in 1894 studied material from the nasal passages of 29 individuals whose duties required their presence for different periods of time

in a room occupied by tuberculous patients. Virulent tubercle bacilli were demonstrated 9 times.

Kälble<sup>10</sup> in 1899 reported the demonstration of tubercle bacilli by guinea pig inoculation tests from the presumably normal bronchial lymph nodes in 2 cases of 23 investigated. One of the positive cases was an adult, 41 years of age, and the other a child 5½ years of age. The material utilized by Kälble was examined grossly and microscopically for lesions of tuberculosis, but no evidence of infection was noted.

Wang<sup>11</sup> quoted a study by Griffith<sup>12</sup> which included material from a series of 61 children who showed at autopsy no morbid signs of a tuberculous infection. The bronchial and mesenteric lymph nodes from each individual, and in one instance the cervical lymph nodes also, were used to inoculate guinea pigs. Material from 5 of the cases produced tuberculosis in the test animals and the infective agent in 3 of these was found to be of the bovine type.

Griffith<sup>12</sup> investigated the infectivity of bronchial and mesenteric lymph nodes of children who failed to show morbid signs of tuberculosis at autopsy. Material from 34 of the cases was studied by inoculation into guinea pigs and the bronchial lymph nodes of 2 of the children were found to contain virulent tubercle bacilli of the human type.

Recently (1938) Saenz and Canetti<sup>13</sup> reported having demonstrated tubercle bacilli in 1 of 14 specimens from apparently normal lungs. They concluded that tubercle bacilli were present but rarely in the tissues of presumably normal lungs.

This résumé of the literature, while not complete, indicates that occasionally tissues such as the parenchyma of the lung or the contiguous lymph nodes may contain virulent tubercle bacilli although morbid signs of the infection are not detectable. However, whether the presumably non-tuberculous parenchyma of the lung does or does not contain tubercle bacilli cannot be established with finality. The technical difficulties presented preclude the examination by any available method of other than a very small portion of the total substance of the lung. That positive results are obtained occasionally from relatively small portions of the organ prompts the suggestion that tubercle bacilli might be demonstrated more frequently if it were possible to use the entire paren-

chyma for test purposes. However, if the lung tissues of clinically and anatomically non-tuberculous individuals do contain tubercle bacilli in any considerable percentage of cases, it is difficult to account for the apparent lack of clinical or morbid signs of disease as a consequence of the presence of such bacteria. It may be, as some contend, that the natural resistance imparted as a consequence of the primary complex is operative and is successful in most instances in preventing the development of foci of reinfection.

It is not surprising that the atmosphere of an environment in which there is a relatively large number of tuberculous individuals should at times contain tubercle bacilli. It has been shown, however (Fishberg,<sup>14</sup> 1932), that in adults such exposure to the infective agent is rarely followed by recognizable evidence of disease. However, in those instances in which the infective bacteria are proved to be present in the tissues with no clinical or pathological signs of tuberculosis, it is conceivable that during life they might constitute a hazard to susceptible individuals.\*

The fact that the 3 cases in our series which yielded positive results represented individuals who were at the time of death inmates of a psychopathic hospital is of interest. Patient 1, who had been confined to the institution for the last 25 years of life, hardly warrants consideration since the cause of death was tuberculous enteritis and peritonitis, which conditions provide sufficient explanation for the presence of tubercle bacilli in the tissues of the lung. This was probably a terminal episode since the lungs were without gross or microscopic signs of recent or active tuberculous infection.

Patient 2, however, from whose tissues tubercle bacilli of the bovine type were isolated, had been committed to the hospital only 9 days prior to death. In this instance the fact that tubercle bacilli were demonstrated in the presumably non-tuberculous parenchyma of the lungs is of less importance than the fact that the tubercle bacilli obtained were bovine rather than human in type. This would seem to be an important observation although the explanation for the possible source of the infection is obscure.

\* Shrewsbury and Barson<sup>5</sup> raised the question of the existence of temporary or permanent "carriers" of tubercle bacilli in instances where there were no clinical signs of tuberculosis but where tubercle bacilli were demonstrated in the sputum by cultural procedures.

In this instance only 1 of the 6 guinea pigs inoculated with material from the lungs and hilar lymph nodes was tuberculous at the time of autopsy, 57 days after injection. The lesions observed at autopsy were definitely characteristic of those of "injection" tuberculosis in the guinea pig and were unlike the morbid changes that occur in tuberculosis of guinea pigs acquired spontaneously. Culturally and pathogenically the organism recovered from the tuberculous spleen of this guinea pig had the features that distinguish the bovine form of the tubercle bacillus.

It should be noted in this connection that the type of the tubercle bacillus recovered from Patient 2 would not have been identified had not cultures from the diseased spleen been attempted. This suggests the desirability of isolating and identifying the provocative agent in every instance where possible in studies of this character. Perhaps if the practice were more universally followed other strains of tubercle bacilli that are presumed to be of the human type might prove to be otherwise.

Patient 3 of our series had been a patient in the psychopathic hospital for 3 months before death. Whether the tubercle bacilli demonstrated were acquired from the environment of the institution or elsewhere is problematic.

The significance of our findings consists, we believe, not in the fact that the tissues from a small percentage of the individuals contained virulent tubercle bacilli, but rather in the fact that in most of the material examined no tubercle bacilli were found. This, we believe, is important and coincides with a rational concept of tuberculosis as the disease occurs in areas where the morbidity from tuberculosis is not high. If the reverse were generally true, one might expect a higher incidence of active infection.

A possible explanation of the differences in our findings and those of Opie and Aronson<sup>1</sup> may be sought in the differences in the concentration of tuberculous infections in the respective environments from which the material in the two studies was obtained. The greater part of our material came from an area where the morbidity from tuberculosis is not high, while that used by Opie and Aronson represented a portion of a population from an area where tuberculosis is, or was, more than commonly present. Furthermore, another factor that may be significant is the fact that there has been a gradual but definite decline in tuberculous

infections in the United States during the decade or more since Opie and Aronson's studies were completed.

### SUMMARY AND CONCLUSIONS

Opie and Aronson having reported the demonstration of virulent tubercle bacilli in the presumably non-tuberculous lung tissue in 15 of 33 bodies examined in Philadelphia in 1927, a comparable study was made of material secured at Rochester, Minnesota. Tissues from 51 unembalmed bodies were utilized for the inoculation of guinea pigs. The age distribution was from 2½ to 93 years with the largest number of cases between 50 and 69 years inclusive. All were white. Thirty-four were males and 17 were females. The bodies selected for the study represented individuals who, with one exception, had died of causes other than tuberculosis. In 12 of the bodies no gross or microscopic evidence of tuberculosis was found, while in 38 there were lesions of latent or healed tuberculosis. In the majority of instances the signs of primary tuberculosis present were those of the primary complex of the lungs.

Material for the inoculation of guinea pigs consisted of what appeared to be non-tuberculous portions of the upper and of the lower lobes of each lung and the apparently non-tuberculous hilar lymph nodes. In all but 3 cases, 3 emulsions of tissue were prepared from each body and were used to inject 6 guinea pigs. A total of 150 emulsions was utilized to inject a total of 300 animals.

Positive results were obtained from only 3 individuals and since in 1 of these the cause of death was tuberculous enteritis and peritonitis, only 2 positive cases need be considered. In 1 case tubercle bacilli definitely identified as bovine in type were obtained from the spleen of 1 of 2 guinea pigs previously inoculated with a composite emulsion prepared from presumably normal tissues from the parenchyma of the upper and lower lobes of the left lung. In another case tubercle bacilli were demonstrated from the hilar lymph nodes.

The results of this study, made of material from an area where the morbidity from tuberculosis is not high, indicate that virulent tubercle bacilli are infrequently present in the presumably non-tuberculous tissue of the lungs of individuals dying of causes other than tuberculosis.

NOTE: We wish to acknowledge the valuable assistance rendered by Dr. A. G. Karlson in certain technical aspects of this study.

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## FIXING AND STAINING METHODS FOR LEAD AND COPPER IN TISSUES \*

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Lead and copper are of great importance clinically, especially lead, owing to their poisonous properties. Unfortunately there are no specific differential stains for these metals in tissues as there is, for example, for iron. Therefore it has not been possible to study them microscopically in direct connection with the lesions they produce. Both metals will stain gray to black if thin pieces of tissue are placed in a solution of hydrogen sulphide, but the test is not at all delicate and microscopically is useless. The object of this communication is to call attention to two simple staining methods which although only partially differential are very delicate and histologically of value.

### STAINS FOR LEAD

*Hematoxylin Stain:* Hematoxylin and its ripened derivative hematein unite with a number of metals to form colored compounds, some of which have been found very useful as stains for nuclei and other tissue elements. As a rule hematein is required to make the stain effective. For this reason an alum hematoxylin solution must be ripened by the aid of light, heat or an oxidizing reagent. For staining lead, hematoxylin itself is essential and ripening of the staining solution must be prevented as far as possible. On this account a solution in dibasic potassium phosphate has been found most useful.

*Fixation:* Tissues to be examined for lead must be fixed in 95 per cent or absolute alcohol. Formalin is worthless and therefore tissues fixed in this reagent in the past are useless.

*Method of Staining:* Stain celloidin sections in the following solution in the paraffin oven (about 54° C.) for 2 to 3 hours, rarely for longer. Paraffin sections are loosened from the slide.

Dissolve 5 to 10 mg. (but not more) of hematoxylin in a few drops of absolute or 95 per cent alcohol and add 10 cc. of a freshly filtered 2 per cent aqueous solution of dibasic potassium phos-

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phate. After staining, wash the sections in several changes of tap water for 10 minutes to 1 hour, dehydrate in 95 per cent alcohol, clear in terpeneol and mount in terpeneol balsam.

*Results:* By this method lead is stained a light to a dark grayish blue, and the nuclei (which owe their staining properties to the presence of metals) take a deep blue color.

Other solutions of hematoxylin ripen so quickly that hematein or some intermediate product is formed and stains the lead brownish instead of blue and are therefore useless.

The best tissue for studying the effect of acute poisoning by lead is obtained by feeding rats with dog chow thoroughly soaked with a saturated (about 1 per cent) solution of lead chloride and giving them the same solution to drink. Pulverized metallic lead and lead carbonate and phosphate may also be used but act more slowly. In 8 weeks the cytoplasm of the liver cells contains numerous small, round and irregularly shaped granules which tend to fuse together to form networks and which stain clear blue by the hematoxylin method given above.

Excellent tissue for study can also be obtained by feeding *Macacus rhesus* monkeys with lead chloride but they are much more susceptible to lead poisoning than rats. A monkey given 25 cc. of a saturated solution daily on its food became partially paralyzed in its hind legs and unsteady on its feet in 8 weeks. Microscopically many liver cells were necrotic, mitotic figures were numerous and small foci of regenerated liver cells occurred here and there. In the old cells were granules of different sizes which tended to fuse together to form networks. Both the granules and the networks stained blue in the hematoxylin solution recommended above. The liver tissue showed all the microscopic changes of an early beginning cirrhosis. A smaller dose and longer time seemed all that was necessary to produce a typical cirrhosis but much time (months or years) would evidently be required.

*Methylene Blue Stain:* Using sections from these same experimental lesions produced by lead, it was found that the granules which stain with hematoxylin stain even more intensely by methylene blue. Staining 10 to 20 minutes in a 0.1 per cent solution in 20 per cent alcohol is sufficient and decolorization in 95 per cent alcohol takes about the same length of time. For microphotographic purposes this stain is of value.

These two staining methods were tried on sections of alcohol-fixed livers from numerous cases of alcoholic cirrhosis. The old hyalin, which is characteristic of this type of cirrhosis, was stained slightly or not at all by both methods, probably owing to the disappearance of the lead. On the other hand, many of the younger regenerated liver cells contained numerous granules and small networks which stained intensely blue and resembled closely those produced in rats and monkeys by acute poisoning with lead.

The methylene blue solution was then tried on paraffin sections of Zenker-fixed livers from rats and monkeys poisoned with lead. The granules and beginning networks stained intensely blue. The same was true of sections of livers from human cases of alcoholic cirrhosis and if the usual staining method of phloxine followed by methylene blue was employed and the methylene blue was allowed to act long enough so as to stain deeply, the old hyalin was colored various shades of red while the granules and beginning networks were stained deep blue.

The probable explanation is that the lead unites with the chrome salt present in the Zenker's fluid and the lead chromate formed is not soluble in the acetic acid and therefore persists.

#### STAINS FOR COPPER

Copper is fixed well by both alcohol (95 per cent or absolute) and neutral formalin, and is stained intensely blue by hematoxylin and by hematein. The simplest method is to use the same solution recommended for staining lead. The iron so commonly associated with copper stains black after alcohol fixation, but light to dark brown after formalin fixation.

Rats were poisoned with copper acetate (normal cupric) by giving them each 2 cc. of a 5 per cent aqueous solution daily on their food. The dosage was a little too large because they began to die after 4 months. Two organs, the liver and the kidney, showed marked lesions. In 2 to 3 months all the liver cells contained small granules which stained intensely blue to blue-black with hematoxylin. After that length of time the liver cells began to undergo necrosis and regeneration took place. The resulting picture after 6 months was very much like that seen in the liver from an early active case of hemochromatosis but the pigment present was copper hemofuscin, not hemosiderin, and the granules

stained blue, not black. The beginning elimination of copper and transformation of hemofuscin to hemosiderin seem to require about 7 months.

The lesion in the kidneys was equally marked. Copper hemofuscin was deposited abundantly in the cells lining the convoluted tubules and caused necrosis. The desquamated necrotic cells filled the lumens of the tubules and active regeneration occurred along the walls.

When the hematoxylin staining method was tried on sections of livers from cases of hemochromatosis it was found that the hemosiderin in the pigment stained black after alcohol, but light to dark brown after formalin fixation. In the islands of regeneration, when present, where the pigment was being laid down and was therefore freshly formed, the granules stained blue to blue-black, strongly suggesting copper.

Frequently inspissated bile was found in dilated bile capillaries. It was colored deep blue by hematoxylin, indicating copper, and clearly explains the manner in which copper gains entrance to gall stones where its presence was pointed out by Schönheimer and others.

In the kidneys from cases of hemochromatosis the lesion was much less marked than in the animals poisoned with copper, but hemosiderin was found in some of the cells of the convoluted tubules and in a few cases casts in the collecting tubules stained blue with hematoxylin, indicating copper.

When the methylene blue stain was used on sections of the experimental lesions produced in rats the copper stained blue but to only a moderate degree. The same was true of the copper in the islands of regeneration in the liver in hemochromatosis. For staining the latter lesions methylene blue has, however, one great advantage. Hemosiderin does not take the stain and therefore appears yellow to light brown. As a result the copper, in spite of its light blue color, shows up quite clearly. When the pigment granules contain both copper and iron they stain green.

#### COMMENT

For many years we have been working primarily on the subject of chronic lead poisoning and its relation to alcoholic cirrhosis, and to a less extent on chronic copper poisoning and its relation

to hemochromatosis. It seems advisable to publish at the present time the methods found useful for fixing these metals, especially lead, in the tissues and for demonstrating them by special stains. Because the staining methods are only partially differential and therefore not diagnostic, they have to be supported by a certain amount of histological and experimental work. As a result a brief summary of our results to date is presented.

Both metals, but especially copper, are very slowly acting, chronic poisons. To produce experimentally all the steps of the various changes which occur in man would require a number of years. In the meantime much valuable pathological material might be lost if not properly preserved. Alcohol is the best fixative for both metals and is absolutely necessary for demonstrating lead. Formalin is about as useful as alcohol for copper. Zenker's fluid is the best fixative for tissues to be studied histologically and preserves both metals but cannot be considered as an ideal fixative for them because the hematoxylin staining method is useless after it. The wisest procedure is to preserve tissues in all three fixatives from all important cases.

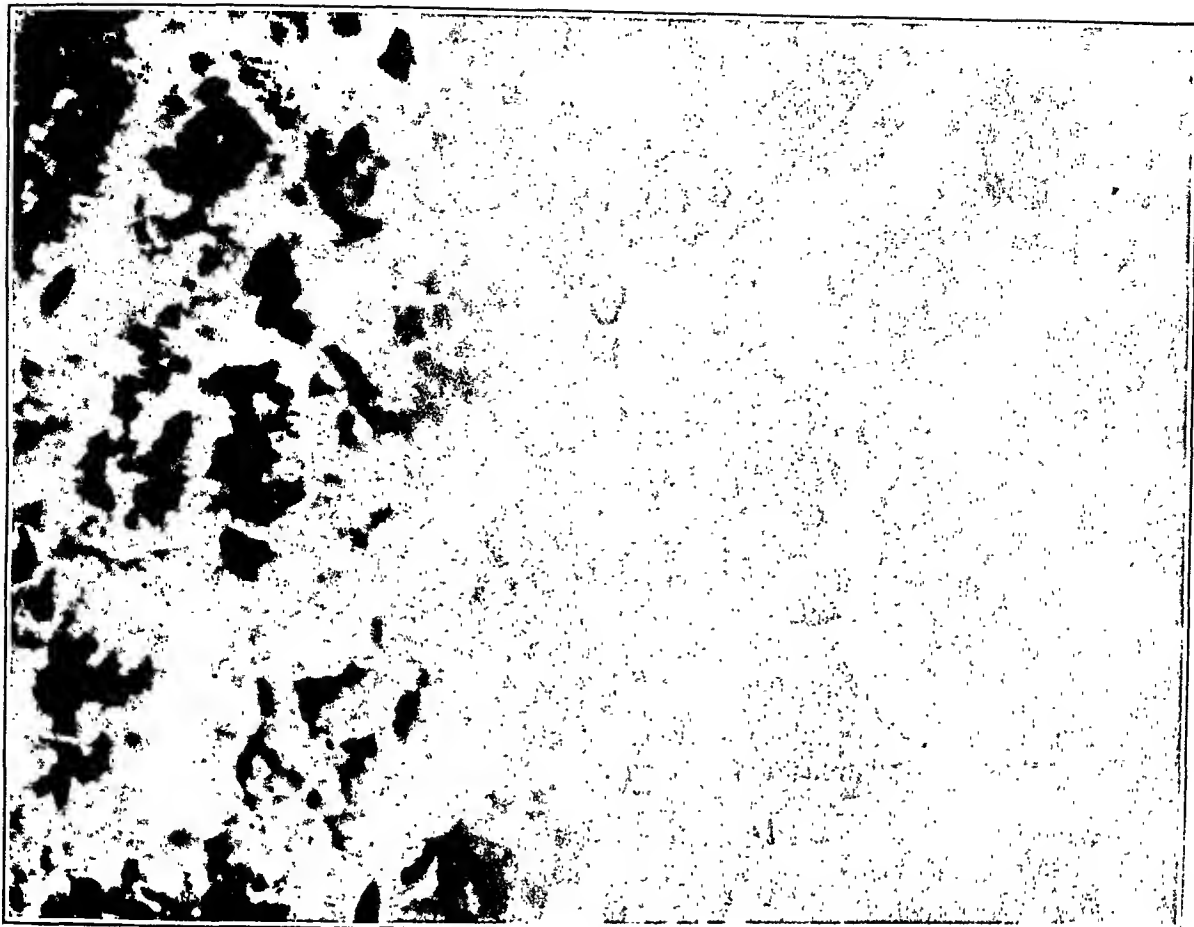
The liver would seem to act as a temporary storehouse for lead which causes little or no evident damage unless the amount absorbed exceeds a certain minimum.

Hyaline bodies were found in the nuclei of liver cells in the monkey and the hog, as in children, but were not found in the rat. They stained blue with hematoxylin like the granules in the cytoplasm of the liver cells but slightly less intensely.

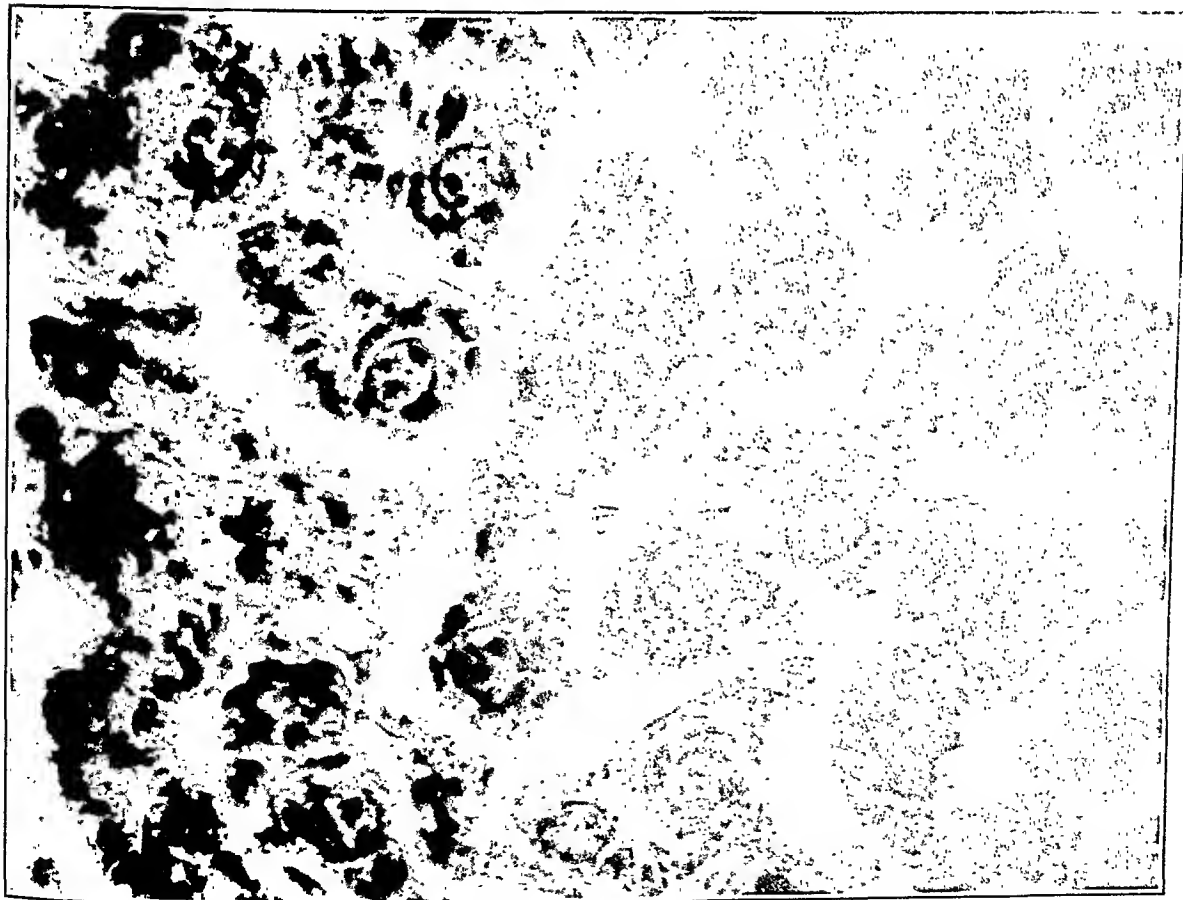
The decision whether or not a case of cirrhosis is due to lead, copper or some other agent must be made by a pathologist using the best differential stains available. The patient may have had hemochromatosis and yet not have imbibed any amount of copper for months or years before death and it might have been largely or entirely eliminated during that time. Under these conditions the chemist is helpless and his results valueless unless his work is controlled by careful microscopic examination to show whether lead or copper is present or not. On the other hand, an individual may have a normal appearing liver and yet may have imbibed a large amount of lead or copper during several months preceding death, and therefore show much of one or the other metal present.

The hematoxylin staining method given here, when applied to





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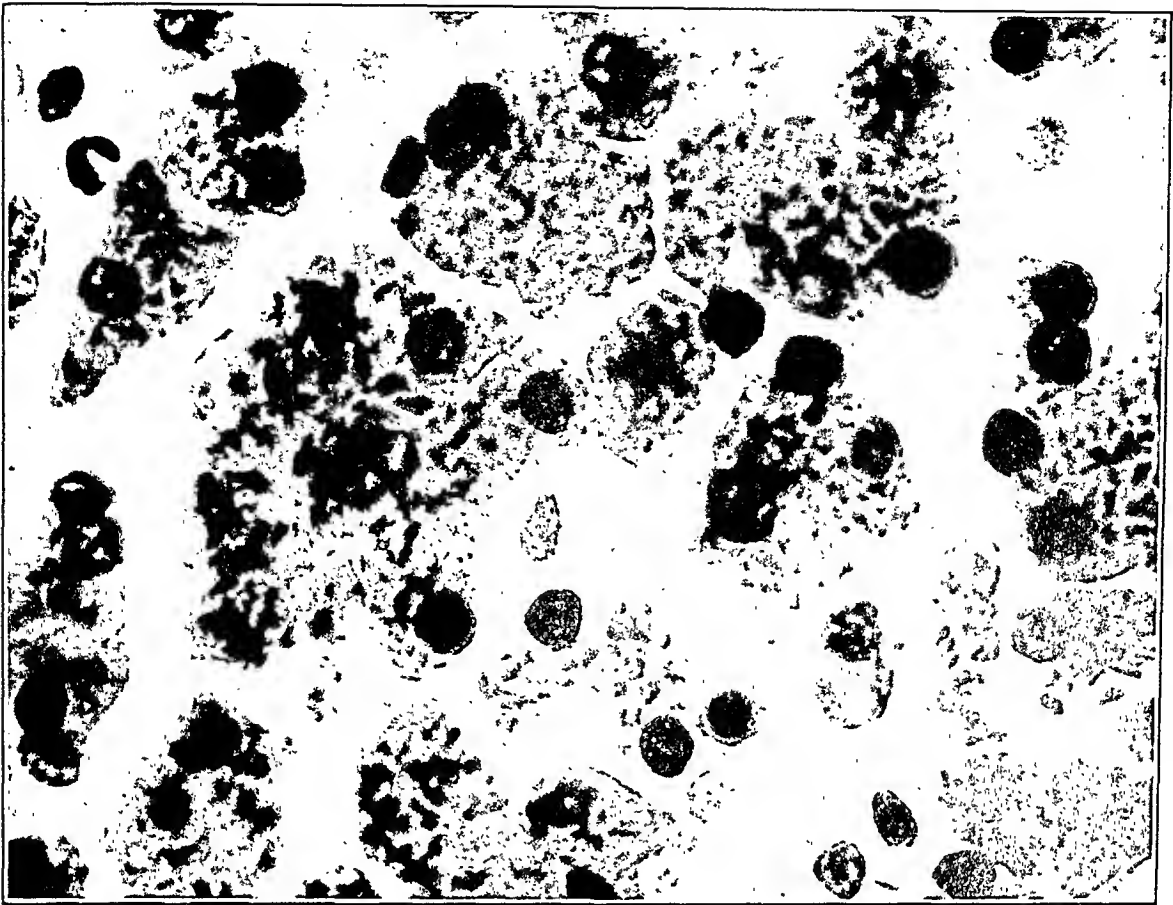
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PLATE 84

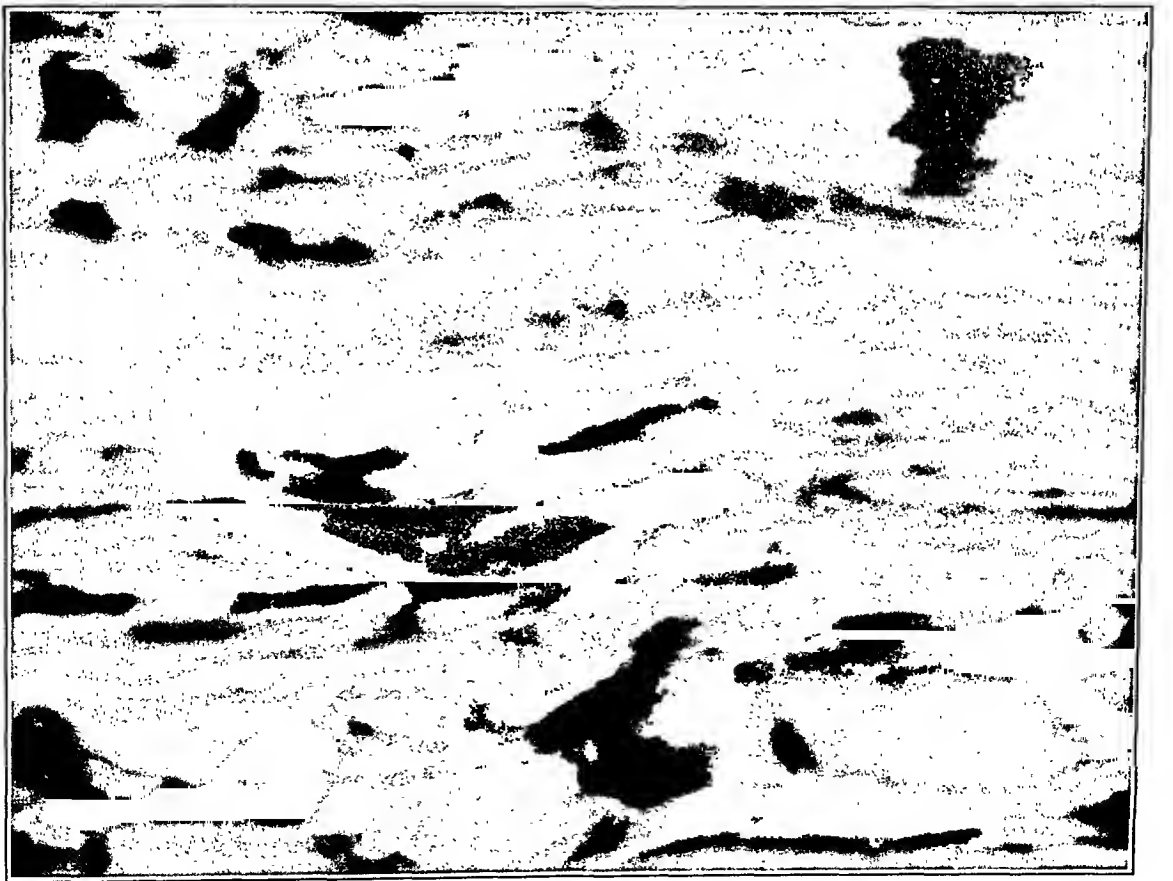
FIG. 3. An island of regeneration from an active case of alcoholic cirrhosis containing much old hyalin. The liver cells are filled with granules and young hyaline networks stained intensely blue. Fixation in Zenker's fluid. Phloxine-methylene blue stain.  $\times 1000$ .

FIG. 4. A normal peripheral nerve showing the myeloaxostroma as stained by the hematoxylin method recommended.  $\times 1100$ .





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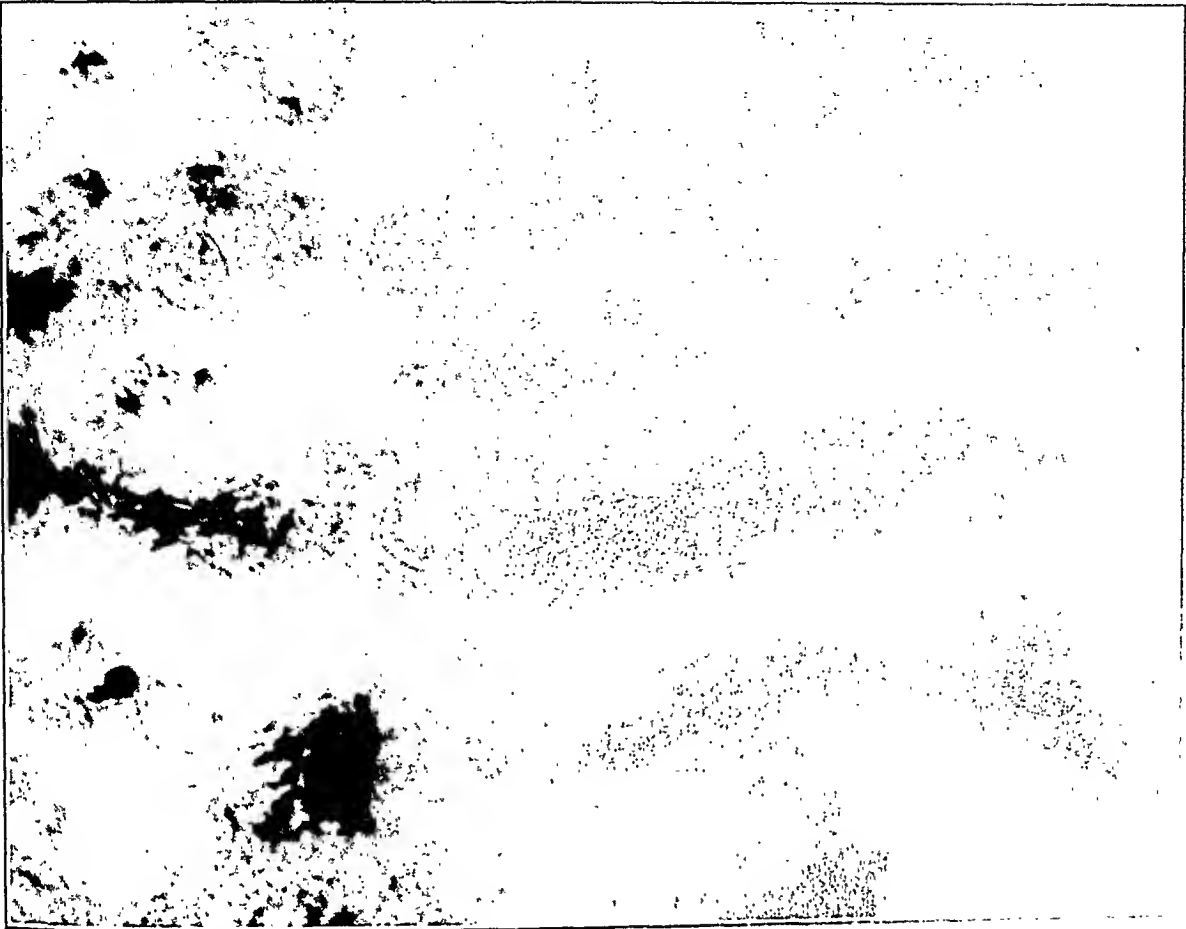


PLATE 85

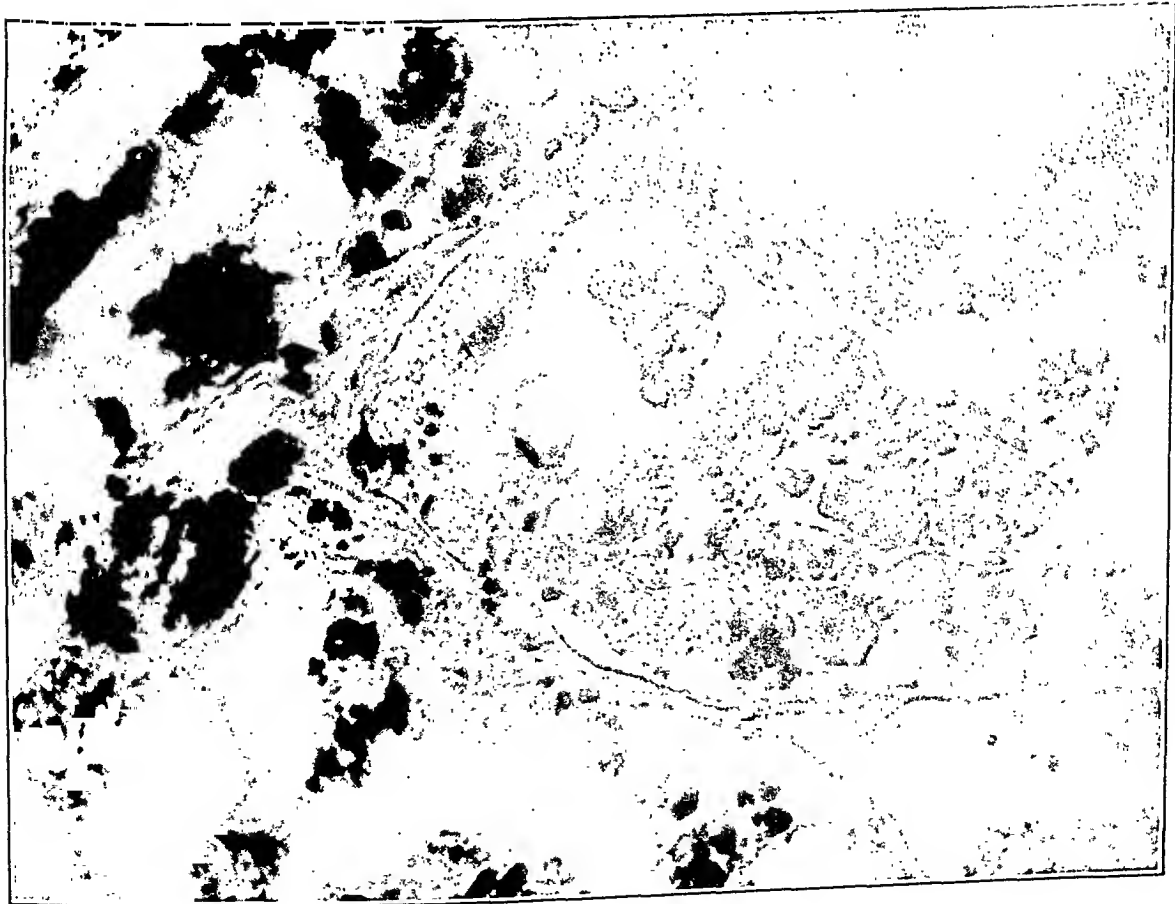
FIG. 5. Liver of a rat fed cupric acetate for 6 months. The liver cells are filled with masses of granules containing copper stained a deep blue by hematoxylin. Fixation in alcohol.  $\times 1000$ .

FIG. 6. Kidney of a rat fed cupric acetate for 6 months. The convoluted tubules are distended with necrotic cells filled with granules containing copper stained deep blue with hematoxylin. Fixation in alcohol.  $\times 1000$ .





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# GIANT CELL FORMATION IN THE TONSILS IN THE PRODROMAL STAGE OF CHICKENPOX \*

## REPORT OF A CASE

T. H. TOMLINSON, JR., M.D.

(From the Division of Pathology, National Institute of Health, Washington, D.C.)

Alagna<sup>1</sup> in 1911 reported the histopathology of the nasal and pharyngeal mucosa and the lymphoid tissue in 8 children dying during the course of measles. He noted in the lymphoid follicles large cells which showed a basophilic cytoplasm and nuclei divided into two or three large masses of chromatin. He also found, without special location, nuclear masses made up of eight to fifteen nuclei lying one on top of another and with borders delineated by a thickened nuclear membrane. It was not possible to differentiate the cytoplasm in these latter structures, which he compared to the nuclei of megakaryocytes.

Next came Warthin's<sup>2</sup> report, published in June, 1931, and Finkeldey's<sup>3</sup> in August of the same year. Since then numerous reports of the occurrence of giant cells in the lymphoid tissue, especially of the tonsils and vermiform appendix, in cases of measles have appeared in the literature. However, there has been no report concerning the presence of similar cells in the tonsils and adenoids in the prodromal stage of chickenpox. The purpose of this paper is to report such a case.

## REPORT OF CASE

*Clinical History:* L. T., a female Navajo Indian, aged 5 years, was admitted to the Southern Navajo General Hospital at Fort Defiance, Arizona, on Feb. 13, 1938, because of an infected lacerated wound of the arm. This infection cleared up and a tonsillectomy was done March 11, 1938, at the father's request. On March 14, 1938, the child developed chickenpox and remained in the hospital until March 31, 1938. There was no record of any other childhood diseases. Previous to the tonsillectomy the patient had been in a ward where several cases of chickenpox had developed.

Histopathological examination of the palatine and pharyngeal tonsils was done on March 29, 1938. There was a moderate, patchy capsular and trabecular scarring. The hyperplastic sur-

\* Received for publication May 22, 1939.

face epithelium showed focal pericellular edema, patchy lymphocytic and neutrophilic infiltration, and occasional collections of pus on the surface. Embedded in this exudate on one tonsil was a mass of fungi. Occasional to numerous scattered tissue mast cells appeared in the capsule and pericapsular fibrous tissue, and in areas extended inward along the trabeculae with occasional cells lying in the adjacent pulp. In one tonsil there was a medium sized area of moderate to marked eosinophilic infiltration of the ill-defined trabeculae and adjacent pulp with occasional cells invading the surface epithelium. Here the capillaries were crowded with neutrophils. Beneath the epithelium there were scattered clumps of occasionally binucleated plasma cells, and in rare areas scattered neutrophils. There was pronounced vascular endothelial swelling. The follicles were large and hyperplastic with huge germinal centers. The reticulum cells of the follicles were swollen but not proliferated and there was much free and phagocytosed nuclear débris. The crypts did not appear to contain an unusual amount of keratin and only occasional clumps of diplococci and short chains of streptococci were seen in the surface exudate and débris.

In addition to these indications of a chronic hypertrophic tonsillitis there were numerous, scattered and grouped, large multinucleated cells. These appeared predominantly within the follicles although many were scattered in the pulp and occasional groups lay just beneath the surface and epithelium of the crypt. Occasionally in the adenoids, but rarely in the tonsils, giant cells were seen in the epithelium. These cells varied from round to oval in shape and usually had a regular outline, although in some instances it was difficult to visualize a definite limiting membrane owing to the dense packing of the nuclei. Often the innumerable overlapping nuclei completely filled the larger cells which were usually seen in the germinal centers. Frequently in the smaller cells there was some peripheral cytoplasm, but it was less abundant than is usually seen in typical foreign body giant cells. At the periphery of the follicles and in the pulp there were occasional, small to medium sized cells showing peripherally placed nuclei with small central zones of cytoplasm. The cells varied greatly in size, the largest measuring 114 by 42  $\mu$ , and the smaller ones being less than 15  $\mu$  in diameter. The nuclei measured from 4.5

to 10  $\mu$  in their long axes and varied in number from three or four to ninety or more. The majority of the cells showed no evidence of degenerative changes, and in these cells the cytoplasm was neutrophilic to slightly oxyphilic, usually finely granular but at times somewhat foamy. However, the cells in the pulp of the adenoids showed a distinctly thready, slightly basophilic cytoplasm. The nuclei, while occasionally oval, were usually round with medium sized chromatin particles radially arranged around a central nucleolus. Progressive stages in degeneration of the giant cells were seen. The nuclei became hyperchromatic and lost their reticular structure while the cytoplasm became more oxyphilic. Finally the clearly outlined cell membrane enclosed a mass of homogeneous, deeply oxyphilic cytoplasm in which were embedded clumps and strands of deeply staining, almost black, irregularly shaped and diffusely distributed nuclear débris. In some instances this débris almost filled the cell, in others it was more densely massed in the central portion, and in still others as much as half of the cytoplasm was still visible as an irregular peripheral rim or as islands distributed in the nuclear material. No phagocytosed nuclear débris, cells or microorganisms were seen within any of the giant cells.

#### DISCUSSION

The giant cells seen in this case appear to be identical with those described in measles. If in this case the association of the giant cells with chickenpox rather than with measles is admitted, one must feel hesitant in accepting their presence as warranting a diagnosis of measles. In rendering the original report on this case to the clinician a probable diagnosis of measles was given, only to have the clinical history and diagnosis of chickenpox reported by him 4 weeks later. Study of additional cases of varicella is, of course, essential before any definite conclusions can be drawn. The demonstration of these giant cells in a second virus disease would seem to warrant further investigation of lymphoid tissue in other virus exanthemas.

#### SUMMARY

An apparently well authenticated case of chickenpox developed in a 5 year old Indian girl 3 days after a tonsillectomy. Histo-

pathological examination of the tonsils showed numerous giant cells seemingly identical with those previously reported as occurring during the prodromal stage of measles.

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2. Warthin, Aldred Scott. Occurrence of numerous large giant cells in the tonsils and pharyngeal mucosa in the prodromal stage of measles; report of four cases. *Arch. Path.*, 1931, 11, 864-874.
3. Finkeldey, W. Über Riesenzellbefunde in den Gaumermandeln, zugleich ein Beitrag zur Histopathologie der Mandelveränderungen im Masern-inkubationsstadium. *Virchows Arch. f. path. Anat.*, 1931, 281, 323-329.

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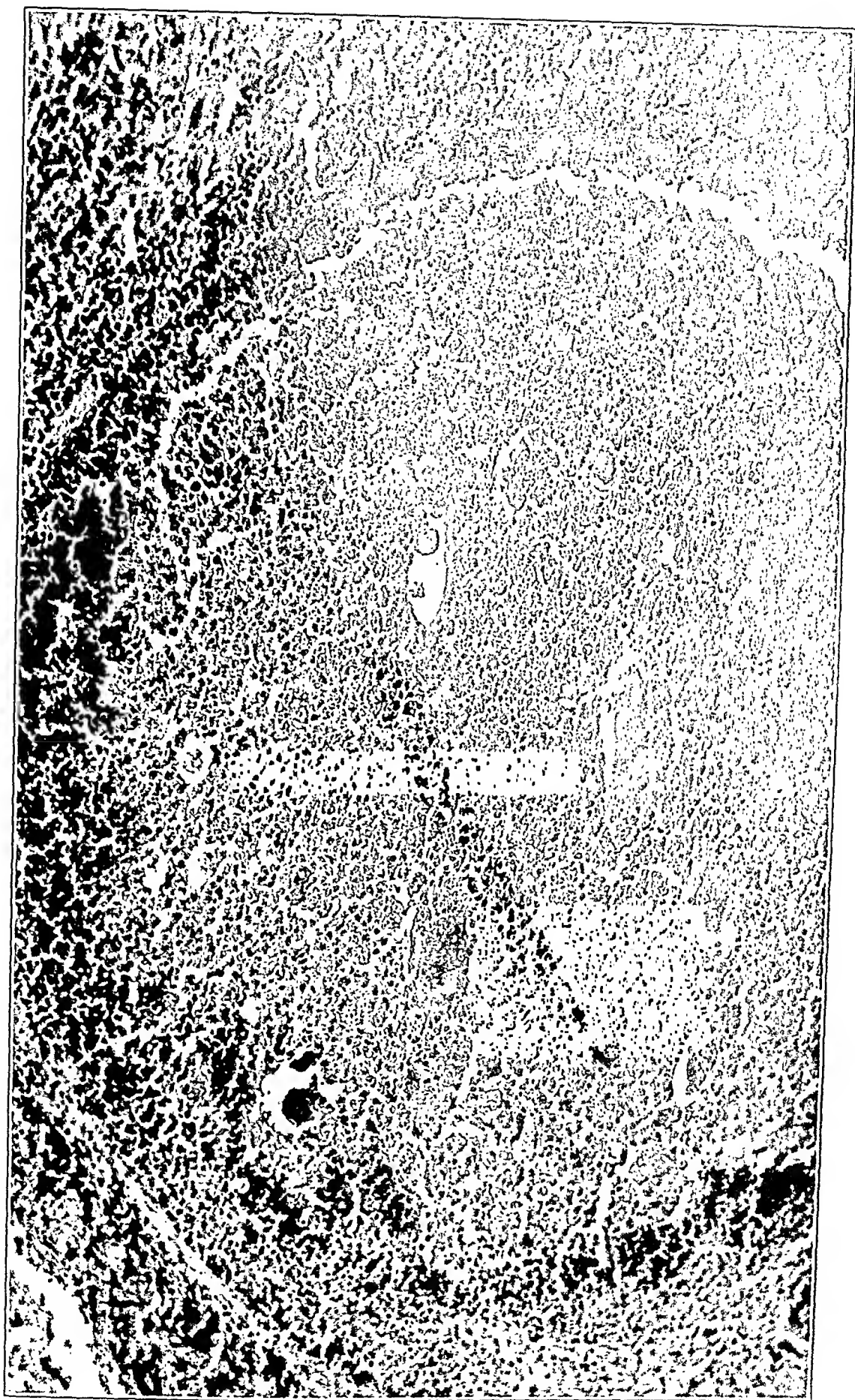
#### DESCRIPTION OF PLATES

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##### PLATE 86

FIG. 1. (N.I.H. 1148.) A large follicle containing numerous giant cells, some of which are degenerating. Hematoxylin-Romanowsky stain.  $\times 100$ .





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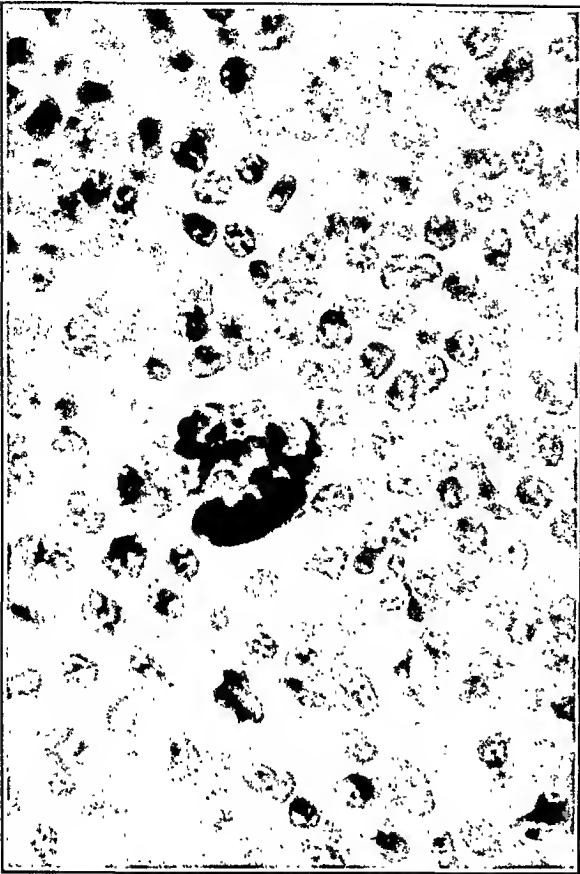
Tomlinson

Giant Cells in Tonsils in Chickenpox



PLATE 87

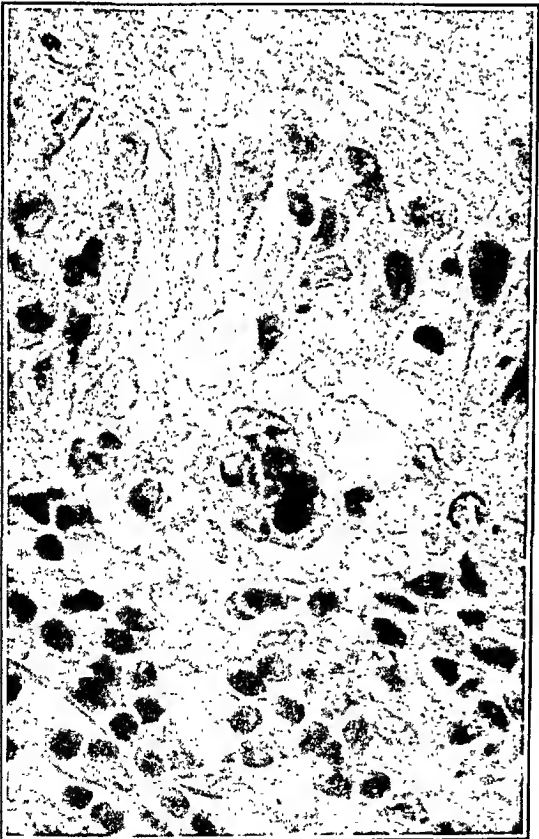
- FIG. 2. (N.I.H. 1152.) A degenerating giant cell at the periphery of a follicle. Van Gieson's stain.  $\times 700$ .
- FIG. 3. (N.I.H. 1149.) A giant cell of the Langhans type in a follicle. Van Gieson's stain.  $\times 700$ .
- FIG. 4. (N.I.H. 1153.) A small giant cell just beneath the epithelium of the pharyngeal tonsil. Van Gieson's stain.  $\times 650$ .
- FIG. 5. (N.I.H. 1150.) A giant cell in the pulp of the palatine tonsil. Van Gieson's stain.  $\times 250$ .
- FIG. 6. (N.I.H. 1151.) A large giant cell measuring 114 by 42  $\mu$ . Van Gieson's stain.  $\times 250$ .



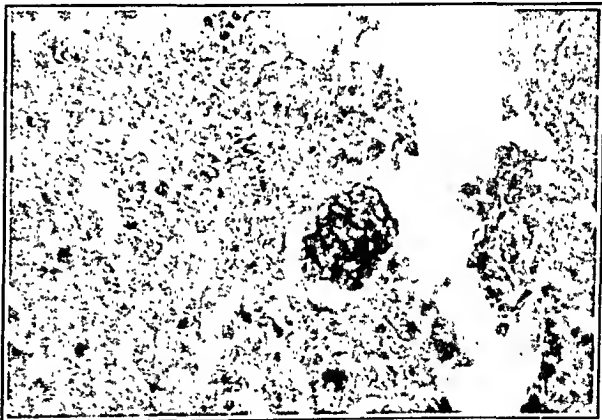
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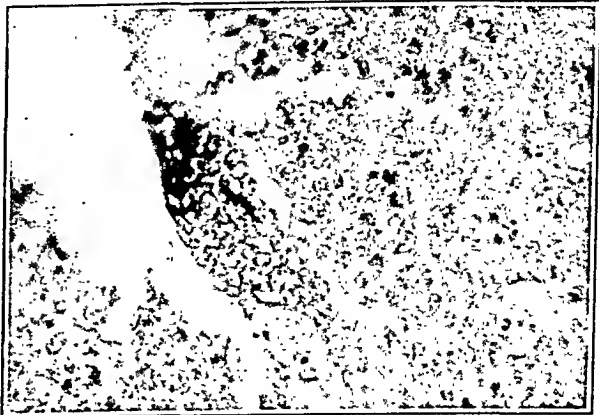
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## TRAUMATIC AUTOTRANSPLANTATION OF SPLENIC TISSUE \*

SAUL JARCHO, M.D., AND DOROTHY H. ANDERSEN, M.D.

*(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City, and the Pathological Laboratory of the Babies Hospital, New York City)*

For several decades it has been known that following severe trauma to the region of the spleen the peritoneum may be found to contain nodules composed of a tissue grossly and microscopically similar to normal spleen. Cases of this type are apparently quite rare, as a survey of the literature shows. Despite this the subject is eminently worthy of study because it involves certain principles of function, growth and transplantation which are among the most fundamental problems of biology.

The earliest well marked case occurring among humans was reported by Faltin.<sup>1</sup>

A boy aged 9 years suffered traumatic rupture of the spleen. Splenectomy was performed. Six years later laparotomy was performed for appendicitis. The peritoneal cavity was found to contain innumerable lentil sized nodules, covered with serosa, and scattered over the large and small intestines. Microscopically these nodules resembled spleen.

Faltin believed that these nodules arose from dormant areas of splenic anlage which as a result of splenectomy had received a stimulus to full development. He felt that the nodules constituted compensation for the splenic tissue removed at operation.

A similar case was reported by Küttner.<sup>2</sup>

An elderly man received bullet wounds of the spleen and colon. Splenectomy and intestinal repair were performed. Four years later the patient died of coronary arteriosclerosis. At autopsy a mass of splenic tissue about 6 cm. in diameter and another about 2 cm. in diameter were found in the left hypochondrium. In addition, innumerable nodules were found over all the intestinal loops. These lesions consisted of splenic pulp, unaccompanied by trabeculae.

This case was repeatedly mentioned in the literature both by Küttner, who performed the original splenectomy, and by Beneke<sup>3</sup> who performed the autopsy.

Oltmanns<sup>4</sup> in a dissertation not accessible to us described a case in which nodules of splenic tissue were found in the peritoneal

\* Received for publication June 6, 1939.

cavity of an individual who had previously undergone splenectomy for traumatic rupture of the spleen.

Von Stubenrauch<sup>5,6,7</sup> has made a series of clinical and experimental contributions to existing knowledge of the subject. He described a case as follows:

A man suffered a severe crushing injury of the trunk. At operation, 36 hours later, huge quantities of blood were found in the peritoneal cavity. The spleen was squeezed and broken, the fragments being about 10 cm. apart. After splenectomy and a complicated postoperative course lasting 5 weeks the patient recovered. He subsequently developed intestinal obstruction. Laparotomy disclosed the presence of innumerable nodules ranging from 0.25 to 0.5 cm. in diameter. These nodular lesions contained trabeculae, sinuses and lymphocytes, but were devoid of lymphoid follicles.

Von Stubenrauch was able to implant autogenous splenic tissue in the peritoneal cavity of dogs, cats and rats. He considered splenic tissue highly susceptible to autotransplantation. He believed, however, that splenic autotransplants tend to regress. He stated that splenic function can be compensated for in three ways: (1) by changes in the bone marrow and lymph nodes; (2) by regeneration of the main mass of splenic tissue; and (3) by the formation within the peritoneal cavity of organs which have a structure like that of splenic tissue. To these structures he gave the name "splenoids." This "splenoid" theory seems to have originated with von Stubenrauch and has been accepted by subsequent authors. The existence or non-existence of splenoids is of great importance and will be discussed in the later pages of the present study.

Von Stubenrauch's last article<sup>7</sup> offers these conclusions: (1) in cases of the type previously detailed a traumatic origin of the peritoneal nodules cannot be assumed; and (2) clinical experimental evidence does not warrant the belief that splenic tissue presents any well marked proliferative tendency.

Küppermann<sup>8</sup> states: "It has not rarely happened that in splenectomized persons who for any reason were subjected to subsequent laparotomies the peritoneal cavity has been found to contain certain small formations which on microscopic examination proved to be accessory spleens." This comment accompanies the report of the following case:

A boy aged 15 years was hurt in a bicycle accident. The spleen was found to be smashed and was accordingly excised. A half year later the patient was

operated on for incisional hernia. The peritoneal cavity contained about 100 brown and red nodules, some of which were about 0.5 to 1 cm. in diameter. On microscopic examination these were shown to consist of normal splenic tissue.

Shaw and Shafi,<sup>9</sup> whose extensive review we have used freely, reported the following case:

An Egyptian male aged 20 years had undergone splenectomy for a traumatic lesion of the spleen. Several years later this patient died in uremia. He was found to have 82 nodules, most of which were in the peritoneal cavity. One nodule was found in the left pleural cavity, 1 on a vertebra, and 1 beneath the capsule of the liver. The nodules contained structures resembling splenic pulp and capsule.

These 6 cases gathered from the literature bear the closest possible resemblance to the following hitherto unpublished cases.

### CASE REPORTS

**CASE 1:** A boy aged 6 years was knocked down by a truck. The wheel of the truck struck the left side of the boy's chest and abdomen but did not run completely across the trunk. The family noticed that the patient was pale and very thirsty. About 16 hours after the accident he complained of pain in the upper left abdominal quadrant, which was aggravated by coughing. The patient was brought to the hospital and laparotomy was performed at once, a ruptured spleen being removed.

The spleen weighed 90 gm. It bore a ragged tear extending half way through the organ from about the middle of the anterior margin. Section of the splenic tissue showed no abnormality except for hemorrhage around the laceration.

Subsequent roentgen examination disclosed the presence of a fracture of the clavicle but no other bony abnormalities. A blood transfusion was given and prompt recovery ensued, the hemoglobin increasing rapidly from 55 to 100 per cent. The healing of the wound was delayed by several stitch abscesses.

During the next 1½ years the child enjoyed good health.

At the end of this period, *i.e.* 2 days before his death, he began to complain of cramp-like pain around the operative scar. He refused food and began to vomit. On admission to the hospital 48 hours later he was in extremis; he had poor color, grunting respiration, and a distended tympanitic abdomen. He died 3 hours after admission. The clinical diagnosis was peritonitis secondary to rupture of the appendix.

*Postmortem Examination:* The changes of greatest interest are in the peritoneal cavity. The peritoneal surfaces bear a little film and there are 200 cc. of amber serous fluid in the peritoneal cavity. Several fibrous adhesions bind the splenectomy wound to various loops of intestine and to the omentum. The largest mass of adhesions is attached to the ileum at a point 62 cm. above the ileo-



CASE 2 \*: A boy aged 12½ years sustained a severe blow on the abdomen while coasting. He then walked about a quarter of a mile to the hospital. He presented the complaint of pain in the left upper abdominal quadrant and in the left shoulder. These symptoms were aggravated by deep breathing. The patient was found to be a well developed boy, pale and ashen. The pulse was small, its rate 132. There was mild tenderness on deep pressure over the left upper abdominal quadrant.

The patient became increasingly pale and dyspneic. At operation, 3 hours after the original injury, about a liter of partly clotted blood was found in the peritoneal cavity. The spleen was deeply lacerated but not otherwise abnormal. It was excised, after ligation of the pedicle. About 450 cc. of blood removed from the peritoneal cavity was strained through gauze, citrated, and injected intravenously. The postoperative course was complicated by an infection of the operative wound and by phlebitis.

Eight years later the patient died of appendicitis and multiple peritoneal abscesses.

*Postmortem Examination:* The autopsy disclosed, in addition to these lesions, approximately 80 nodules ranging from about 1 mm. to 3 cm. in diameter. These were situated in the peritoneum, omentum and diaphragm, and along the greater curvature of the stomach. There were numerous adhesions about the splenic bed. The splenic artery and vein had become recanalized and were found to have sent off branches to some of the nodules.

*Microscopic Examination:* Sections of 10 nodules were available for study and may be described collectively. The capsules are found to be thin. In most instances typical trabeculae are absent. In 1 nodule a band of connective tissue extends downward from the capsule into the pulp and contains a relatively large blood vessel; the pattern is that of a trabecula. For the most part the nodules are composed of tissue which in appearance and arrangement resembles the red pulp of spleen. In one instance a small group of lymphocytes resembles a crude follicle. The sinuses of the pulp are readily discerned as almost all are engorged. Scattered siderophages are encountered.

A minute nodule found in the subserosa of the stomach differs from the foregoing in certain details. This nodule is encased in a dense collagenous capsule. The pulp consists of lymphoid tissue, with sinusoids and one well marked follicle containing an arteriole. In a section stained with silver an argyrophilic reticulum is dem-

\* This case is reported through the kindness of Dr. Paul Klemperer of the Mount Sinai Hospital.



onstrated. The relation of the nodule to the serosa is not clearly determinable from this section.\*

In view of the fact that blood removed from the peritoneal cavity at the time of splenectomy had been injected intravenously, it is interesting to note that there were no splenic nodules in the lungs.

CASE 3†: This patient was severely injured in an automobile accident several years ago. As a result of this accident she has a scar in the skin of the left hypochondrium. A pelvic operation performed several years after the original trauma revealed the presence of nodules scattered over the intestines. A nodule removed for biopsy was shown to consist of tissue resembling spleen. At the present time it is still not known whether splenectomy was performed in this case.

In each of the 10 foregoing cases there was a clear history of trauma.

Completeness requires the mention of certain additional cases in which there was no definite history of antecedent trauma. The first of these is Albrecht's widely quoted case.<sup>10</sup>

A man aged 25 years died of nephritis. Scattered throughout the peritoneal cavity, omentum, diaphragm and pelvis were about 400 nodules which Albrecht regarded as accessory spleens. The principal spleen was about 2.5 cm. in diameter and was adherent to the diaphragm and omentum. At its lower pole there were several packets of partly adherent accessory spleens. The left kidney was flattened, shrunken and scarred.

In his comment on this case Albrecht first refuted Toldt's hypothesis that the coelomic epithelium of the mesogastrium might possess spleen-forming potentialities at many points. Such an explanation would not account for the lesion of the left kidney. Albrecht preferred to believe that during intrauterine life a strong and presumably mechanical disturbance had acted upon the region of the spleen and left kidney. As a result, the kidney was almost completely destroyed, while the splenic anlage was broken into countless pieces, which were scattered over the peritoneal cavity

\* During a recent visit to a neighboring republic one of us (S. J.) saw a patient whose history was almost identical with that of the patient just described. A young male, evidently about 17 years of age, had undergone splenectomy for traumatic rupture of the spleen. Several years later he was operated on for appendicitis. Innumerable nodules resembling small spleens were found in the peritoneum and on the intestines. It is to be hoped that this case will ultimately be published in detail by the patient's physicians.

† Through the courtesy of Dr. Henry Horn the authors have recently learned of this case which is to be published by Dr. J. H. Buchbinder and Dr. C. J. Lipkoff of the Knickerbocker Hospital.

and in the omentum and became implanted there. These implants would of course be carried along in the wake of subsequent embryonal development and would suffer the same alterations of position and arrangement as the omental and peritoneal reflections. Albrecht abstained from stating specifically that the abnormal process was due to trauma. He judged that the cause of the condition was unknown but was presumably mechanical in nature.

Albrecht's case has been mentioned repeatedly in the literature. It was discussed at a meeting of the Gesellschaft deutscher Naturforscher und Ärzte. On this occasion Beneke<sup>3</sup> presented Küttner's aforementioned frankly traumatic case. Beneke remarked that in his opinion Albrecht's case may have been traumatic in origin. In the discussion Sternberg<sup>11</sup> said that in Albrecht's case trauma had been considered but that no definite evidence thereof had been found. Paltauf<sup>12</sup> stated that he also was familiar with Albrecht's case and believed the cause to be trauma.

The case reported by Schilling<sup>13</sup> (quoted by Shaw and Shafi<sup>9</sup>) likewise presents no definite history of trauma.

The patient was a woman aged 47 years who had died of carcinoma of the uterus. In the abdominal cavity there were 42 nodules ranging from about 1 mm. to 2 cm. in diameter. The main spleen was deeply involved in adhesions, sharply angulated, and divided into three lobes by deep incisures. The left kidney measured only 2 by 1 by 0.2 cm. and was deformed. Almost all the nodules were found to have normal splenic structure.

After an extensive discussion of the embryology of the peritoneum Schilling concluded that some unknown principle, acting during intrauterine life, had scattered part of the splenic anlage over the peritoneal cavity. This unknown agent was considered insufficient to stop the further development of the principal spleen but had impaired the progress of the left kidney.

The following case was reported by Tedeschi.<sup>14</sup>

A girl aged 15 years died of meningitis. The spleen was found to be ectopic and somewhat atrophied, and bore many depressed scars. The transverse colon was displaced obliquely toward the left. The spleen was situated deep in the left side of the peritoneal cavity. Along the splenic vessels there were about 50 accessory spleens, and 2 more were found in the gastrocolic omentum. There were many lymphoid formations in the liver.

A similar case was reported by Jolly.<sup>15</sup>

A girl aged 15 years had vague abdominal pains and nausea and was thought to have appendicitis. At operation the peritoneum was found to contain huge numbers of round and ovoid nodules ranging from 0.1 to 0.5 cm. in diameter. These nodules contained well marked malpighian corpuscles and were believed to consist of splenic tissue. The condition of the main spleen was not ascertained. The history made no mention of trauma.

Of these cases all but 4 present a clear history of antecedent trauma. In 4 cases (Albrecht,<sup>10</sup> Schilling,<sup>13</sup> Tedeschi,<sup>14</sup> and Jolly<sup>15</sup>) the clinical histories make no mention of trauma. The facts which these cases provide are fortified by certain observations derived from a study of lower animals.

According to Ceresole,<sup>16</sup> Zambecari<sup>17</sup> in 1680 was the first to observe nodular formations after experimental splenectomy. From the quotation given by Ceresole there is some doubt that the nodules described by Zambecari were similar to the nodules which form the subject of the present discussion. More precise descriptions were given by many more recent authors, such as Tizzoni,<sup>18-23</sup> Abelous, Argaud and Soula,<sup>24</sup> Amormino,<sup>25</sup> and others.

Guerrini<sup>26</sup> reported the case of a dog aged 8 years. Ten months prior to its death this animal had suffered a severe physical injury which had been followed by prolonged illness and recovery. Ultimately the animal died of a diarrheal disease. Nodules of splenic tissue were found widely scattered in the peritoneal cavity. The spleen contained a hemorrhagic cyst but had no wound on its surface. Consequently Guerrini concluded that the numerous splenic nodules were not implants. He inferred that the splenic cyst had produced splenic insufficiency; as a result of this deficiency a swarm of preëxisting accessory spleens had been stimulated to hypertrophy.

The present authors disagree with Guerrini's interpretation. The so-called hemorrhagic cyst was very probably the result of laceration of splenic pulp—the so-called incomplete rupture of the spleen, the pathogenesis of which is well described by Englmann and Hitzler.<sup>27</sup> It is not unlikely that a splenic scar was present near the cyst but was small and escaped detection. The peritoneal nodules thus could easily have been produced by implantation of splenic tissue scattered at the time of the splenic injury. Moreover, in the recent experiments of Bloom and Taliaferro<sup>28</sup> less than 1 per cent of experimental infarcts of the spleen

were followed by permanent scarring. It is therefore possible that in Guerrini's case the animal originally suffered an incomplete rupture of the spleen and splenic pulp was scattered in the peritoneal cavity. The deeper portions of the splenic rupture then healed incompletely and formed a cystic and hemorrhagic lesion, while the surface of the spleen regenerated completely.

The coexistence of blood cysts of the spleen and intra-abdominal nodules was also reported by Binet.<sup>29</sup>

Tizzoni,<sup>18</sup> who did much of the important earlier work in this field, reported that in 1 dog he found 262 nodules of splenic tissue in the peritoneal cavity. The main spleen was extensively scarred by "chronic interstitial splenitis."

Professor Jarmai<sup>30</sup> of the veterinary college at Budapest reported the case of a terrier which died a few days after an injury. The spleen was found to be broken completely in half; the larger half bore two scars. More than 400 nodules resembling spleens were found growing in the peritoneal cavity. Subsequently it was learned that in previous years the dog had suffered two severe injuries, including a fall from a window.

Jarmai mentions more briefly the case of another dog which had had an injury 1 year before death and which was ultimately found to have 483 accessory spleens. Köves<sup>31</sup> (quoted by Jarmai) reported a less conclusive case occurring in a pig. This paper has not been available to us.

By reason of their uniformity the foregoing cases are readily summarized. In certain individuals who had undergone splenectomy for traumatic rupture of the spleen, the peritoneal cavity was subsequently found to contain a smaller or larger number of nodules composed of a tissue closely resembling spleen. Four additional cases presented nodules of this type without data as to previous trauma; in 3 of these 4 cases the main spleen was small and shrunk or distorted; in 2 of the 4 cases the left kidney was similarly affected. Parallel observations in animals are also cited.

In explanation of these phenomena several different hypotheses suggest themselves. We may at once dismiss the possibility that the nodules *precede* the splenic trauma and hence are unrelated thereto. This view — which at all events does not apply to the cases in humans — depends on the fact that small animals, espe-

cially dogs, are occasionally found to have unexplained splenic tissue in the peritoneal cavity. This tissue, in accordance with arguments to be presented (*vide infra*), is more probably attributable to the myriad injuries which small animals inevitably suffer.

A second hypothesis proposes that the nodules are formed by the enlargement of preëxisting lymphoid tissue or splenic anlage. It has been thought that splenectomy might stimulate such structures to proliferate and to form nodular masses.

This opinion was held by many of the earlier students of the problem, such as Foà,<sup>32</sup> Faltin,<sup>1</sup> and Capelli<sup>33</sup>; it receives its support in part from analogy. Thus it is stated that splenectomy may be followed by general enlargement of lymph nodes.<sup>34</sup> One might also cite the fact, mentioned by Schmidt,<sup>35</sup> that in mice splenectomy is followed by the development of nodular formations (lymphomas?) in the liver. The observations of De Kock<sup>36</sup> in ruminants are likewise of interest in this connection.

A serious objection to the "compensatory hyperplasia" hypothesis is the fact that nothing recognizable as splenic anlage or lymphoid tissue is known to occur in such places as the intestinal subserosa or the diaphragm, which are among the commonest sites of the nodules. It must also be remarked that *the occurrence of widely disseminated splenic nodules in any considerable number has never been reported after splenectomy in cases of non-traumatic disease of the spleen. This suggests that the determining factor is not splenectomy but trauma.*

Specific data confirming this statement have been obtained from the autopsy files of the Presbyterian Hospital and of the Babies Hospital. In the last 2000 autopsies at the Babies Hospital there were 9 cases in which splenectomy had been performed for various reasons. The interval between splenectomy and death ranged from a few hours to 9 years. The only case in which the operation had been performed because of traumatic rupture of the spleen was that reported in this paper (Case 1) and it was likewise the only case in which there were nodules widely spread over the peritoneal surfaces. In 2 other cases there were accessory spleens in the usual site in the fat near the bed of the spleen. During the last 10 years there have been 2605 autopsies at the Presbyterian Hospital; there had been a previous splenectomy in 16 of

these cases; the interval between operation and death varied from 2 weeks to 15 years. In no instance in the Presbyterian autopsy series was splenectomy performed because of traumatic rupture, and in none of these cases was the splenic tissue found elsewhere than in the bed of the spleen. Accessory spleens were found in 3 cases.

A third hypothesis is of even greater theoretical interest. This is the dictum that the nodules are formed by the peritoneum, perhaps under the stimulus of splenectomy.

It has been difficult to discover who first presented this concept. Apparently one of the earliest was Toldt, whose opinion Albrecht<sup>10</sup> quotes (without adequate bibliographic reference) and rejects. Toldt is quoted as having said that the splenic anlage arises by a special alteration of the coelomic epithelium. This alteration ordinarily occurs in a single area but under exceptional circumstances might occur in a number of unconnected foci and thereby generate any given number of unconnected splenic nodules. As has been stated, Albrecht felt that such an explanation could not account for concomitant lesions of the left kidney; he assumed instead that during fetal life a strong and presumably mechanical disturbance had struck the region of the spleen and left kidney, scattering the anlage of the former and damaging the anlage of the latter. Albrecht's explanation must be considered a closer approximation to the truth.

Another early study of the subject was that made by Griffini and Tizzoni<sup>37</sup> in 1883. These authors observed that partial splenectomy in dogs was followed by the development of nodules in the peritoneum. They concluded as follows: "These observations confirm once again that the omentum and the folds of the peritoneum have the property of giving rise to 'new productions' of splenic parenchyma." Similar opinions were expressed independently by Tizzoni.<sup>23</sup> Certain serious flaws in the work of Tizzoni have been pointed out by Meyer.<sup>38</sup>

Faltin's<sup>1</sup> views have already been mentioned. This observer felt that splenectomy stimulated the peritoneum to regain its alleged former power of creating splenic tissue. In other words, dormant areas of splenic anlage were awakened to compensatory activity.

It is in the writings of von Stubenrauch<sup>5, 6, 7</sup> that the theory of

peritoneal origin reaches its fullest development. Von Stubenrauch stated that after excision of the spleen restoration occurred in three ways: (1) by changes in the bone marrow and lymph nodes; (2) by regeneration of the spleen; and (3) by the new formation in the peritoneum of organs resembling spleens. To the latter, which are the nodules under discussion, von Stubenrauch gave the name of "splenoids." Accordingly the entire conception of the peritoneal genesis of splenic nodules can be not inappropriately designated "the splenoid theory."

Von Stubenrauch performed a large number of successful implantations of autogenous splenic tissue in various experimental animals. These experiments resulted in the formation of nodules resembling spleens. Von Stubenrauch concluded that implanted splenic tissue ultimately regressed. He felt that the nodules found in the aforementioned human cases had been created by the peritoneum. Von Stubenrauch's views have been handed down in the literature<sup>39</sup> and seem to have acquired a permanent niche. In Herfarth's review<sup>40</sup> the splenoid theory is considered "possibly correct but by no means conclusively proven." The most recent general treatise on the spleen, that by Klemperer,<sup>41</sup> states: "Clinical and experimental observations show that the loss of splenic tissue can be compensated for in various ways (von Stubenrauch, 1920; Herfarth, 1926): (1) by hypertrophy of splenic tissue left behind at operations; (2) by hypertrophy of accessory spleens (Morrison *et al.*, 1928); (3) by autotransplantation of pulp particles (Kreuter, 1920); and (4) *by formations of spleen-like structures originating from the peritoneum (splenoids).*" (Italics ours.)

This abstract is given in full because it shows that the "splenoid" theory has been accepted by eminent authorities and has won a degree of credence which the facts may or may not warrant. The doctrine that spleen-like structures may be formed from the peritoneum is of fundamental biological importance and deserves complete reinvestigation. Studies now in progress by one of us (S. J.) will, it is hoped, contribute new data toward the elucidation of this problem.

From these considerations it is clear that the "preëxisting nodule hypothesis," the hypothesis of compensatory hyperplasia, and the so-called "splenoid theory" all present certain virtues and

certain inadequacies. One theory remains to be discussed — that the nodules represent implants of splenic tissue scattered throughout the peritoneal cavity as a result of laceration or rupture of the main spleen.

Autoplastic transplantation of splenic tissue has been successful in the hands of many experimenters (Ehrhardt,<sup>42</sup> Manley and Marine,<sup>43</sup> Marine and Manley,<sup>44, 45</sup> von Stubenrauch,<sup>5</sup> Kreuter,<sup>46</sup> Koppányi,<sup>47</sup> Jolly and Lieure,<sup>48</sup> Putschar,<sup>49</sup> and Fujigaki<sup>50</sup>). Genetic aspects of splenic transplantation have been studied by Bittner<sup>51</sup> and by Little and Johnson.<sup>52</sup> Manley and Marine<sup>43</sup> found that spleen autotransplants with considerable difficulty, as compared with thyroid, parathyroid, ovary or adrenal cortex. Marine and Manley<sup>45</sup> found that in rabbits removal of the spleen provides a powerful stimulus to the growth of transplants; this opinion is not universally accepted. Silberberg<sup>53</sup> made autotransplants in guinea pigs and observed that lymphoid and hematopoietic tissue in general have no marked resistance to transplantation.

Perla and Marmorston-Gottesman<sup>54</sup> observed a striking difference in the regenerative capacity of autoplastic splenic transplants in rats as compared with rabbits. Marine and Manley<sup>44</sup> had shown that in the adult rabbit splenic autotransplants would grow in the absence of the main spleen but frequently remained small or even were resorbed. Perla and Marmorston-Gottesman found that in the rat splenic autotransplants would grow even in the presence of the spleen and in its absence would undergo marked hypertrophy. Detailed microscopic studies of autoplastic splenic transplants have recently been published by Perla.<sup>55</sup>

The extensive experiments of von Stubenrauch have been discussed above. He succeeded in obtaining splenic autotransplants yet felt that these were subject to regression and that permanent nodular formations were produced by the peritoneum.

Ehrhardt,<sup>42</sup> using rabbits, obtained successful autoplastic transplants of pieces of spleen and also of whole spleen.

The experiments of Kreuter<sup>46</sup> require detailed consideration. Working with rhesus monkeys, Kreuter found that total splenectomy produced no compensatory formations. Partial splenectomy produced a few nodules resembling accessory spleens. If the spleen was excised and its pulp was smeared all over the peri-



toneum, widely scattered nodules were later found. Kreuter concluded that these nodules could be explained only as the result of trauma and had nothing to do with compensatory hyperplasia or atavism.

Despite Kreuter's controlled and seemingly decisive experiments, Herfarth<sup>40</sup> in a subsequent review concluded that the possibility of autotransplantation (in humans) had not been established beyond cavil but was plausible. This conclusion is a good example of the unwillingness which many of the earlier authors have manifested toward accepting the existence of spontaneous autotransplantation. Herfarth's attitude toward the splenoid theory has been discussed above.

Even more significant was the work of Putschar.<sup>49</sup> This experimenter used the rat, which does not often have accessory spleens. He placed only 3 or 4 implants in each animal. These implants were relatively large (3 to 5 mm.). Some were inserted intraperitoneally, others subcutaneously. The resultant nodules resembled spleens and contained follicles. In 12 rats all implants but 2 were accounted for. Putschar concluded that the nodules could not be regarded as either enlarged hemolymph nodes or as newly created peritoneal "splenoids," since such an explanation could not account for the nodules that had been formed in the subcutaneous tissues.

In view of the work of Kreuter and of Putschar the splenoid theory would seem greatly weakened. Further studies are clearly necessary. Even now it is scarcely possible to avoid the conclusion that the intraperitoneal nodules found after splenectomy in cases of splenic rupture are due to the scattering of particles of pulp throughout the peritoneal cavity. Such scattering is undoubtedly assisted by the profuse hemorrhage which accompanies splenic rupture. This explanation has the merit of simplicity and the support of experimental observation. It obviates the necessity of von Stubenrauch's awkward and otherwise poorly supported assumption that the peritoneum is capable of manufacturing splenic tissue.

#### SUMMARY AND CONCLUSIONS

1. Two cases are presented in which individuals who had undergone splenectomy for splenic rupture were subsequently found

to have numerous nodules of spleen-like tissue scattered throughout the peritoneal cavity.

2. Mention is made of 8 additional cases gathered from various sources, and of 4 cases in which no definite history of trauma was available. In 2 of these the spleen and left kidney were shrunken, scarred and distorted. Several analogous cases in animals are quoted from the literature.

3. The experimental evidence is summarized and it is shown that splenic tissue is susceptible to autoplasmic transplantation.

4. The "splenoid" theory is described and is shown to be a gratuitous assumption inadequately supported by evidence.

5. It is concluded that the aforementioned nodules, found in the peritoneum and omentum of individuals who have previously undergone splenectomy for traumatic rupture of the spleen, are due to autoplasmic transplantation of particles of spleen torn loose from the main body of splenic tissue and disseminated in part by the aid of hemorrhage.

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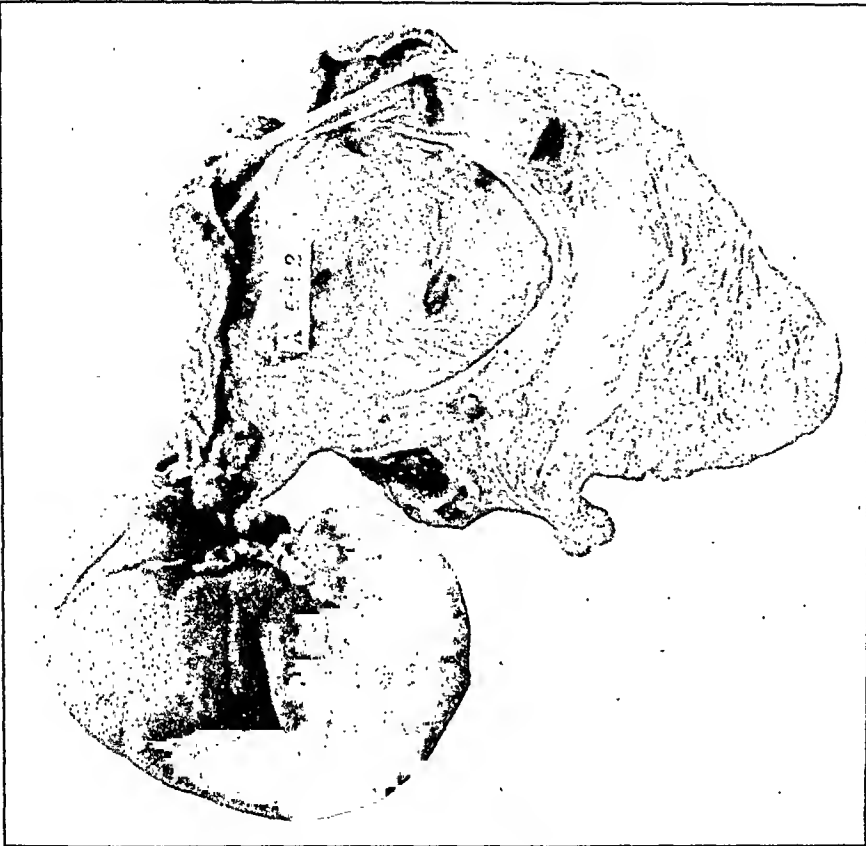
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## DESCRIPTION OF PLATES

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### PLATE 88

- FIG. 1. Case 2. Splenic implants on the anterior wall of the stomach.
- FIG. 2. Case 1. Splenic implants on the pelvic peritoneum.
- FIG. 3. Case 1. Accessory spleens in the omentum.



2



1

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PLATE 89

FIG. 4. Case 1. Splenic implant on the diaphragm.

FIG. 5. Case 1. Malpighian corpuscle in a splenic implant.

FIG. 6. Case 2. Nodule in the wall of the stomach.

FIG. 7. Case 2. The nodule illustrated in Fig. 6 is shown here in greater magnification. Part of a malpighian follicle and its arteriole are shown.



4



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# A COMPARISON OF THE EFFECTS OF ANTERIOR PITUITARY HORMONE ON SKELETAL TISSUES OF YOUNG AND MATURE GUINEA PIGS \*

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Anterior pituitary hormone exerts a growth-promoting effect on the euhyaline cartilage of immature guinea pigs and also produces certain degenerative changes in this tissue.<sup>1,2</sup> These effects were observed in experiments in which anterior pituitary of cattle was implanted, as well as in those in which injections of acid extract of this gland were given. After the demonstration of this action of anterior pituitary hormone on the skeleton of immature animals, it appeared to be of interest to investigate the response of mature skeletal tissues to the same stimuli and, if possible, to correlate them with the changes which normally take place in these tissues with advancing age, particularly since Loeb and his coworkers<sup>3,4,5</sup> have shown that hormones may call forth alterations in the stroma of the thyroid and the mammary glands of guinea pigs and of the vagina and the uterus in mice.

## MATERIAL AND METHODS

Thirty-eight guinea pigs were used in these experiments which were divided into two series. The first series consisted of 16 animals weighing about 400 gm. at the beginning of the experiment. Seven of these animals received 2 cc. each of acid extract of anterior pituitary gland of cattle daily for periods of 4, 15, 30 and 60 days. Two animals received implants of one-half of a gland of fresh anterior pituitary of heifer on 2 consecutive days and were sacrificed on the 4th day. Two guinea pigs received daily implants of one-half of a fresh gland of anterior pituitary of heifer on 4 days and were killed on the 5th day. Two animals also received four implants of one-half of a gland of anterior

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pituitary of heifer on 4 consecutive days after the glands had been subjected to immersion in urea for 15 hours and in glycerine for 24 hours previous to implantation<sup>6</sup>; these animals were also killed on the 5th day. Three guinea pigs of corresponding weight served as normal controls.

In the second series, consisting of 22 guinea pigs, with an initial weight of 750 to 900 gm., larger amounts of pituitary substance were administered, in accordance with the greater weight of the animals. Thirteen animals were injected daily with 4 cc. of acid extract of anterior pituitary gland of cattle intraperitoneally for periods of 4, 5, 6, 7, 10, 14, 30, 45 and 60 days. Two additional animals received daily implants of one-fourth of a gland of fresh anterior pituitary of heifer on 2 consecutive days and were killed 2 days after the second implant had been made. Two guinea pigs received the same amount of fresh anterior pituitary gland daily on 4 consecutive days and were sacrificed on the 5th day. Five animals served as normal controls.

At autopsy the bones were removed for study. Since in the heavy animals our usual technic of incomplete decalcification proved to be a very slow process, some of the bones were decalcified completely in 5 per cent nitric acid. In all other respects the technic employed was the same as in our former investigations.<sup>2</sup>

### OBSERVATIONS

The animals weighing 400 gm. stood the injections of acid extract of anterior pituitary gland well, without showing any marked impairment of their general health, although within the first 10 to 15 days a loss in weight amounting to approximately 10 per cent of the initial weight occurred. But in the animals that were kept alive for periods of 1 and 2 months the weight was soon restored to the initial level, and during the 2nd month of the experiments a gain in weight of about 20 per cent was noted.

The experiments in the second series, however, presented some difficulties, inasmuch as the animals weighing 750 to 900 gm. exhibited a very low resistance to the extract. Their weight fell rapidly, sometimes as much as 15 to 20 per cent within the 1st week of treatment. Some animals had, therefore, to be sacrificed prematurely. Those that survived continued to lose weight,

and at the end of the experiments losses of 35 to 45 per cent were noted.

## MICROSCOPIC EXAMINATION

### *I. Normal Animals*

*Cartilage: (A)* In healthy guinea pigs weighing about 400 gm. and approximately 3 to 4 months old the epiphyseal line was still patent. The cartilaginous ground substance was dense and fibrillar; in some instances collagenous formations had been laid down in this ground substance. The arrangement and distribution of the rows of cartilage cells were less regular than in immature animals. The various cell types, however, were still readily recognizable. The resting cartilage cells were small and flattened, the columnar cartilage cells were fairly numerous, and the replacement of the hypertrophic cartilage cells by bone proceeded in the normal way. In some instances there was evidence of beginning ossification of the epiphyseal line; degenerated cartilage cell columns were replaced by localized wedge-like bony plugs containing remnants of former cartilage cells and horizontal cracks; or here and there a cartilage cell was seen being directly converted into an osteocyte.

In the cartilaginous covering of the joint the sliding, transitional and pressure zones were composed of their characteristic cellular constituents. The layer of the hypertrophic cartilage was almost ossified. In the cartilaginous part of the ribs some hyalinization of the intercartilaginous matrix was observed; the cartilage cells, particularly in the central portions, revealed a slight tendency to undergo either atrophy or hypertrophy, both of these processes being followed by degeneration and solution; but usually only a single cell or isolated cell groups were affected.

*(B)* In normal healthy guinea pigs weighing about 800 gm. the condition of the epiphyseal cartilage was influenced by the age of the animals. In some cases the epiphyseal line of the tibia was very narrow, but it still contained remnants of columnar and especially of hypertrophic cartilage cells which underwent calcification and ossification, and considerable amounts of stroma consisting of collagenous or osseous material were seen. In some instances bundles of parallel thick fibers appeared in the substance separating the cartilage cells, a condition typical of the so-called

asbestos transformation. This condition was found when the guinea pigs were approximately 8 to 12 months old. However, in animals that were in the 2nd or 3rd year of life the upper tibia showed only islands of euhyaline cartilage cells, which indicated the site of the original zone of ossification. As a rule progressive ossification and infiltration by lymphoid marrow had led to a fusion of the diaphysis and epiphysis. In circumscribed, centrally located areas the cartilage of the ribs had undergone retrogression; it was liquefied and cysts had formed in some instances; or the retrogressed parts had been partly replaced by hyaline, calcified or osseous material. The older the animals, the more advanced the retrogressive changes. The demarcation between the cartilaginous and osseous parts of the ribs was sharp. There was no evidence of an invasion of the cartilage by bone marrow. The cartilaginous ground substance of the covering of the joint was acidophilic and hyalinized. The cartilage cells were in a resting stage. Occasionally, in very old guinea pigs, some cells tended to undergo degeneration.

*Periosteum and Bone Marrow:* With progressing age the periosteum and the interstitial connective tissue of the bone marrow increased in amount and became denser. The fibers became hyalinized. In some circumscribed areas the lymphoid cells of the marrow, particularly in the epiphysis, had been gradually replaced by fat tissue. In the diaphysis these alterations were less accentuated. Cells acting as osteoblasts and as osteoclasts were present, indicating that appositional and resorptive processes still took place in mature and in old animals.

## *II. Implants of Anterior Pituitary Gland and Injections of Extract of Anterior Pituitary Gland of Cattle into Young and Old Mature Guinea Pigs*

*(A) Animals Weighing 400 Gm. at the Beginning of the Experiment.*

*Cartilage:* After intraperitoneal injections of 2 cc. of acid extract of anterior pituitary gland of cattle daily on each of 4 consecutive days, processes of softening, swelling, vacuolization and liquefaction of the cartilaginous ground substance of the epiphyseal line were observed. The fibrils were loosened and torn apart. Owing to deposition of calcium in and around such de-

generated areas the epiphyseal zone assumed a darker stain with hematoxylin. The resting and particularly the columnar cartilage cells underwent retrogressive changes, the outlines of the cells becoming more and more indistinct and the nuclei pyknotic. Ultimately these cells disintegrated or became calcified. In some cases only single cells or individual cell groups degenerated; in other instances larger areas of the epiphyseal line were destroyed. Side by side with these degenerative alterations of the cartilage cells growth processes took place. The resting and the columnar cartilage cells proliferated by way of amitosis or, here and there, by mitotic division. The degenerative changes were essentially the same as those seen in guinea pigs weighing 180 gm. injected with 1 cc. of acid extract for 4 consecutive days, although they were somewhat more extensive. On the other hand, the tendency to calcification and proliferation seemed to be less marked here than in the immature guinea pigs. At this early stage there was only a suggestion of the formation of incubator capsules in the chondrophyte; retrogressive and proliferative processes in the ribs and joints were slight or lacking altogether. The lesions obtained after four implantations of one-half of a gland of anterior pituitary of heifer on each of 4 consecutive days were comparable to those seen after four injections of extract, but the effects of two implantations of one-half of a gland of heifer were less pronounced. No difference in the action of fresh glands and of those in which the thyroid-stimulating hormone and the atresin had been destroyed by the *in vitro* treatment of the glands with urea and glycerine<sup>6</sup> could be established.

With an increasing number of injections the degenerative changes in the cartilaginous ground substance receded gradually, whereas the proliferative processes became more marked. After 1 month of treatment the epiphyseal cartilage cells were arranged in regular rows and were more numerous than in the control animals. The epiphyseal line was still patent; it maintained its normal structure and there was none or only a slight evidence of osseous closure of the epiphyseal disc, as seen ordinarily at this stage. In the chondrophyte, cartilaginous brooding capsules appeared. In the joint the cartilaginous covering was thickened; the cartilage cells of the transitional and pressure zones, in which ordinarily the long axis was in a horizontal direction, assumed a



perpendicular arrangement and took on a darker stain with hematoxylin; they proliferated freely by means of amitotic division. Simultaneously the cytoplasm became lighter and enlarged and the nuclei were surrounded by an alveolar space. In some cells atrophy had taken place; in others, and in particular in hypertrophied cells, the nuclei had become pyknotic and the cytoplasm had liquefied and finally dissolved. In addition, capillary loops from the bone marrow perforated the bony border lamella and penetrated into the cartilage (Fig. 1). The ground substance was diminished and hyalinized. In the ribs, particularly in their central portions, similar changes occurred, leading to the formation of minute gaps in the cartilage or to the appearance of hyalinized asbestos fibers.

After 2 months of treatment closure by ossification of the epiphyseal zone had set in, but the epiphyseal line was still wider and the number of the cartilage cells greater than normal. In the joint degenerative processes of the cartilage cells became more accentuated and minute irregularities of the joint surface were noted. In the ribs larger cysts were seen filled with mucoid and amorphous material. The line of demarcation between cartilage and bone, however, was sharp.

*Periosteum and Bone Marrow:* The condition of the osseous tissue was determined by the behavior of the cartilage and the connective tissue in the periosteum and the bone marrow on the one hand, and the balance between appositional and resorptive processes in trabeculae and the compact bone on the other. In the connective tissue a loosening, swelling and tearing apart of the fibrils took place in the early stages. Some reticulum cells of the bone marrow enlarged and assumed a spheroidal and later a polyhedral shape. They appeared honey-combed, the vacuolar spaces being filled with a pale staining clear material (Fig. 2). These cells in which the nuclei were pushed toward the periphery, because of increased cellular pressure, resembled xanthomatous cells and showed all transitions between the latter and ordinary reticulum cells or typical fat cells. They were arranged in islands.

Connective tissue cells along the compact bone and the trabeculae became converted into ameboid epithelioid cells; they were arranged either in a bead-like manner, apparently participating

in the apposition of bone, or underwent fusion and formed multinucleated giant cells. The connective tissue cells, particularly in the subepiphyseal layer of the bone marrow, proliferated and took part in the formation of a fibrotic collagenous tissue which replaced the ordinary lymphoid structure. A similar proliferation in the periosteum led to a thickening of the latter tissue and to the production of precartilage and typical cartilage cells. Following the administration of anterior pituitary extract over a period of from 1 to 2 months, an extensive layer of cartilage cells in the periosteal tissue, situated in the middle and in the upper third of the tibia, was found, and in some places hypertrophied cartilage cells invaded the bony substance. This cartilage underwent hypertrophy and retrogression similar to the cartilage in other regions of the skeleton.

*(B) Animals Weighing 800 Gm. at the Beginning of the Experiment.*

*Cartilage:* The cartilage cells of the joints and of the ribs responded more readily and intensely than the epiphyseal cartilage, which reacted only if large amounts of the hormone had been administered over long periods of time. These changes consisted of a slight hypertrophy and increase in the number of the cartilage cells, mainly in the chondrophyte and in the lateral parts of the epiphyseal zone, which occurred even in instances in which the epiphyseal line was almost completely ossified. An examination of joints and ribs furnished further information as to the action of the hormone. After four implants, or after four injections of the extract, the cartilage cells of the joint, which under normal conditions were resting, proliferated very slightly. The fibrils of the cartilaginous ground substance were loosened and swollen. With increasing doses they disintegrated. The growth processes in the cartilage cells became more accentuated. After 2 to 4 weeks of administration of the extract the cartilaginous covering of the joint was thickened. The cartilage cells of the transitional and pressure zones multiplied and four or more cells were surrounded by one capsule. Subsequently the proliferating cartilage cells underwent hypertrophy. The more the cells enlarged, the more indistinct became the structural details of the nucleus, which finally disintegrated and was destroyed; degenerative changes (karyor-

rhesis and karyolysis) predominated over the growth processes. In some instances the polyhedral cartilage cells assumed a spindle shape and resembled ordinary fibrocytes. The connective tissue cells of the synovial membrane participated in the proliferative processes. In the ligaments, near their insertion, where under normal conditions cartilage cells are found occasionally, an increase in number and size of these cells was observed. The latter likewise underwent retrogressive changes. After 2 months of injections of the extract circumscribed necrotic areas (Fig. 3) and ulcerations of the surface of the joint (Fig. 4) were seen. In some places a pronounced overgrowth of the cartilage cells was present. This pathological tissue, however, showed a marked tendency to disintegration. Degenerated masses accumulated in irregular clumps and advanced toward the surface of the joint where they were cast off. Capillary loops from the bone marrow and the synovial membrane invaded the newly formed tissue. Due to a combination of the proliferative and retrogressive changes, villi or adhesions between the synovial membrane and the cartilaginous covering of the joints were produced (Fig. 5). With increasing amounts of extract, both proliferation and degeneration of the cartilage of the ribs became proportionately more pronounced. However, here as well as in the joints the retrogressive changes predominated. Capillary loops and connective tissue from the marrow invaded the zones of newgrowth and helped to organize the necrotic cartilaginous masses. Thus, an irregular and variegated appearance of the epiphyseal line and the cartilage layers resulted. A fibrous vascularized tissue developed in the normally liquefied and cystic central portions of the ribs. In other places calcification and ossification of the degenerated material were seen.

*Periosteum and Bone Marrow:* At early stages the fibrils of the interstitial connective tissue of the bone marrow and the periosteum became swollen and torn apart, and liquid accumulated in the interstices between the fibers. At later stages, however, a shrinkage of the fibrils was noticeable; their density increased and they underwent hyalinization and sclerosis. Associated with these alterations the following changes in the various connective tissue cells were noted, especially in the marrow of the epiphysis in areas adjoining the insertion of the ligaments: (1) an edematous

swelling led to the appearance of a soft myxomatous tissue containing a limited number of star-like fibrocytes with elongated branching processes; (2) the fibrocytes became converted into precartilaginous and into typical euhyaline cartilage cells embedded in intercellular substance; and (3) a proliferation of fibrocytes resulted ultimately in the development of a dense fibrous tissue.

The first named reaction was usually seen at early stages of the injections of extract. The capillaries were enlarged and slight to moderate extravasations of blood could be detected. The edematous condition of the tissue became more and more pronounced (Fig. 6). Owing to increased pressure, a thinning and absorption of the preëxistent trabeculae and of the bony border lamella of the joints occurred.

The second type of alterations, which led to the appearance of cartilaginous islands within the marrow, was encountered at later stages. In the peripheral parts of such cartilaginous areas ordinary fibrocytes were present, which toward the center gradually assumed the shape of precartilaginous, and finally of typical cartilage cells, thus indicating a direct conversion of fibrocytes into cartilage. In the portions most centrally located, probably due to insufficient nourishment, the cartilage secondarily underwent degeneration and liquefied (Fig. 7). In other cases the cartilage cells invaded and helped to dissolve the osseous substance.

The third type of change was likewise seen after the administration of the extract over long periods of time. In this type the proliferation of fibrocytes went hand in hand with the production of dense collagenous fibers, and it was a dense fibrocytic tissue which participated in the destruction of preëxisting bony material (Fig. 8). The resorption of bone as well as the retrogressive processes in the bone marrow led in some instances to the production of at first small, and later medium sized and large cysts.

As seen also in the young mature guinea pigs in localized areas, in particular in the epiphysis, a conversion of reticulum cells of the bone marrow into pseudoxanthomatous and fat cells was observed. However, in contrast to the changes seen in young animals, no appreciable growth of cartilage cells in the periosteal tissue was noticeable in this series.

## DISCUSSION

In mature guinea pigs anterior pituitary hormone exerts both growth-promoting and degenerative effects on the cartilage, as is also the case in immature guinea pigs. The proportion of these two effects, however, depends on the age of the animals and on the amount of responsive tissue present. In animals weighing 400 gm. the epiphyseal cartilage is almost completely preserved and still possesses the ability to grow under normal conditions. After administration of anterior pituitary hormone it reacts in very much the same way as the cartilage of immature guinea pigs, namely, with degeneration and proliferation. The proliferative process may be so strong that the tendency to osseous closure of the epiphyseal disc, which ordinarily becomes manifest at this age, may be overcome for a certain time. This effect is, however, not indefinitely maintained. During the 2nd month of treatment osseous closure of the epiphyseal zone sets in, either because the stimulus has become less effective or because the growth capacity of the cartilage is exhausted.

In the joints proliferation of the cartilage is quite pronounced, especially in the transitional layer, whereas degenerative processes are less accentuated. Taken together with the capillary loops advancing from the bone marrow, a condition is created which closely resembles the arthropathic lesions of the acromegalic type which we have reported previously.<sup>7</sup>

In guinea pigs weighing 750 to 900 gm., after treatment with anterior pituitary hormone a corresponding reaction of the epiphyseal cartilage takes place, but it is more or less rudimentary inasmuch as in these animals body growth has ceased and the structure of the epiphyseal zone, which is characteristic in young animals, has now disappeared. Therefore, the cartilage of the joint provides a more suitable test object for the present study. Some proliferation of the cartilage cells still occurs in the early stages of the experiments. However, degeneration predominates after 4 or more weeks of treatment, although considerable proliferation of the cartilage may be present even at this stage. But it is very likely that the overgrowth on the surface of the joint represents in part at least a non-specific regenerative process which is not primarily due to the action of the hormone. As to the speci-

ficity of these lesions, it must be stated that while superimposed mechanical factors and regeneration may always play a rôle, both the growth-promoting and the degeneration-producing actions of the anterior pituitary hormone create a condition highly favorable for the initiation and the further development of pathological changes. The severe arthropathic changes which were produced by the anterior pituitary hormone in old animals are comparable to those taking place in arthritis deformans in man.

The alterations in the ribs, which we observed after administration of anterior pituitary extracts, are the result of the same fundamental processes. Degeneration of cartilage, which to some extent is seen also in normal old animals, is greatly enhanced under these experimental conditions, but whereas ordinarily the degenerated areas become calcified to a large extent and only a little ossification occurs, under the influence of the extract a pronounced organization of necrotic material by connective tissue and capillaries originating in the bone marrow takes place.

According to the proportion of proliferative and degenerative changes taking place at the various ages under the influence of anterior pituitary extract, the following gradations can be made.

1. In immature guinea pigs weighing up to 200 gm., degeneration of the cartilage occurs but is transitory. Proliferation is marked and persists as long as it is not overtaken by ossification, which is also increased. Fibrous changes in the bone marrow are only slight. There exists, however, a tendency of the capillaries of the bone marrow to corrode the bony border lamella of the joint and to produce initial stages of arthropathic lesions of acromegalic type.

2. In young mature guinea pigs weighing up to 400 gm., proliferation is about as strong as in immature animals and the retrogressive changes become more accentuated, indicated especially by more extensive arthropathic changes in the joint. The capillaries of the bone marrow show increased resorptive activity and the connective tissue undergoes more fibrosis as compared with immature animals under the corresponding conditions.

3. In older mature guinea pigs weighing up to 900 gm., the retrogressive changes predominate over the proliferative changes. Diffuse degenerative alterations of the cartilage are called forth by the extract and, in cooperation with processes of organization

and regeneration, arthropathic lesions of a deforming type result. The tendency of the bone marrow to undergo fibrosis and degeneration is more pronounced than in the first two groups.

We may then conclude that the action of an anterior pituitary hormone interferes with the normal balance between cells and intercellular ground substance of the cartilage. This interference manifests itself in two different directions. If the hormone is allowed to act on young cartilage, the cells of which possess a certain potency to grow, the proliferation of these cells is increased. But if the cartilage cells have only very little growth tendency, as is the case in older animals, the growth stimulation is less prominent than the occurrence of degenerative changes. With special reference to the process of ageing of cartilaginous tissue this would mean that by the application of hormone preparations, ageing might be delayed at certain stages of development, whereas in older animals it is accelerated by these means. The histogenetic mechanism underlying these processes is complicated by the participation of the highly stimulated connective tissue in different parts of the skeleton. This actively growing tissue, which itself may undergo secondary changes, such as mucoid degeneration and cyst formation, under certain conditions causes solution processes and thus affects the structure of the cartilage of the ribs and joints, as well as of the osseous cortex and the bony trabeculae.

#### SUMMARY AND CONCLUSIONS

In the euhyaline cartilage of sexually mature guinea pigs anterior pituitary extract of cattle causes both proliferative and retrogressive changes similar to those found in immature guinea pigs. With increasing age of the animals the tendency of the hormone to produce proliferation of the cartilage decreases, whereas the tendency to call forth degeneration increases. The retrogressive changes increasing with advancing age consist of liquefaction and formation of cysts in the cartilage, as well as of ulcerations on free surfaces of the cartilage of the joint. Regenerative growth of the cartilage is stimulated even in old animals by injections of anterior pituitary extract. Organization of the areas of degeneration takes place by means of ingrowth of vascularized connective tissue, or by ossification, or by both of these

processes. Thus, an anterior pituitary hormone is able to produce in older guinea pigs severe arthropathic lesions, which, once they have been initiated, may be aggravated by mechanical factors in association with non-specific regenerative processes. These various factors may stimulate the growth of connective tissue, which is able to dissolve and replace preëxisting bone. In the epiphysis the rarefaction of bone, followed by retrogressive changes in the connective tissue which had invaded and replaced the bone, may give rise to the formation of cysts.

NOTE: The authors wish to express their gratitude to Dr. Leo Loeb for his advice and interest in their work.

We are indebted to Mr. S. J. Hayward for the microphotographs.

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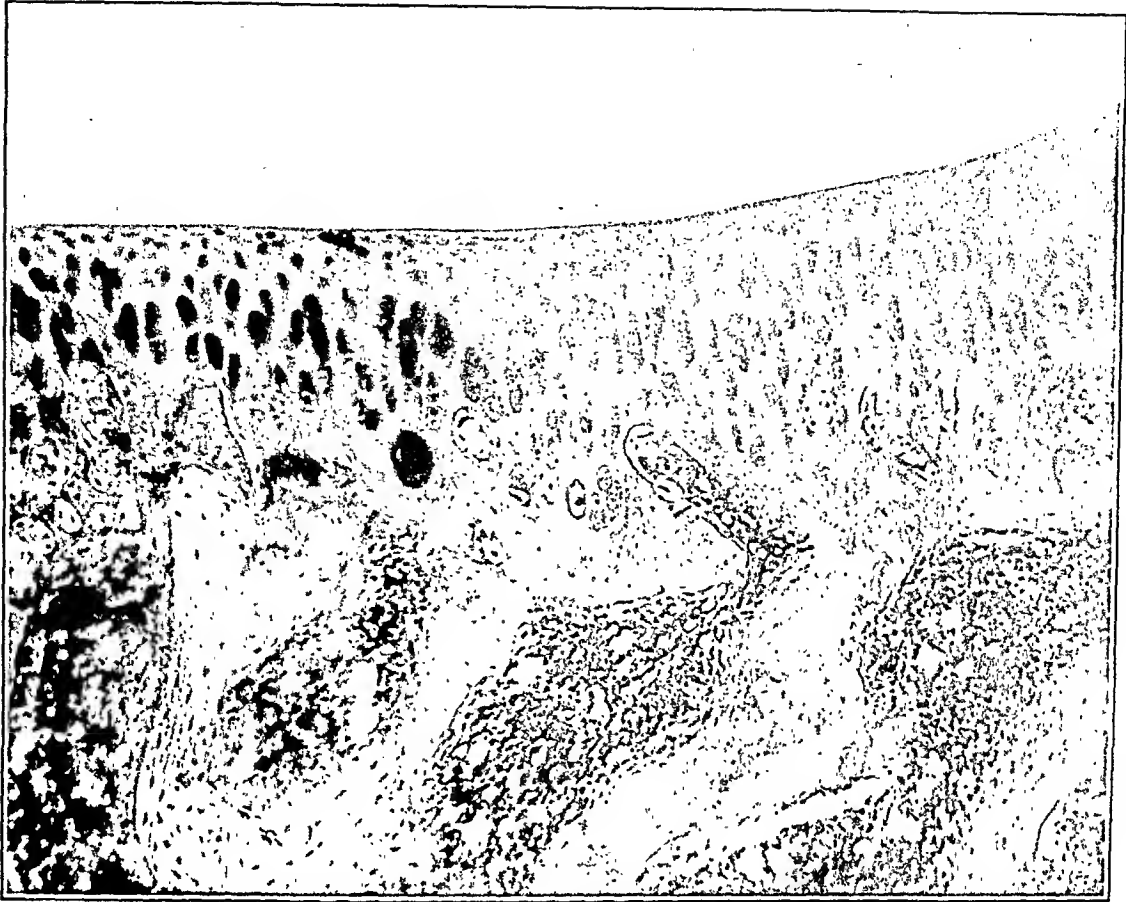
## DESCRIPTION OF PLATES

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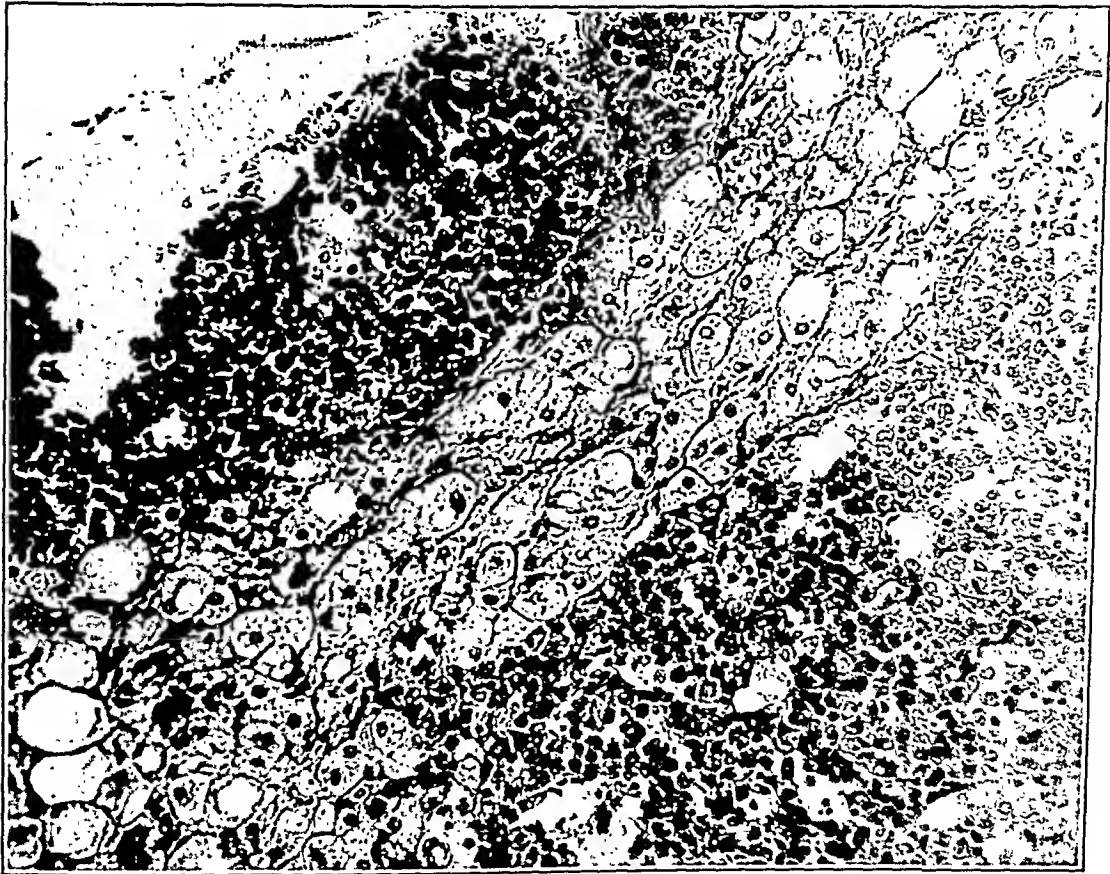
### PLATE 90

FIG. 1. Surface of the knee joint of a guinea pig weighing 380 gm. at the beginning of the experiment. This animal received 2 cc. of acid extract of cattle anterior pituitary gland daily for a period of 30 days. Hypertrophy and hyperplasia of the cartilaginous cell layers is present. Some degenerated cells are to be seen. The capillary loops coming from the bone marrow perforate the bony border lamella and invade the cartilage of the joint.  $\times 120$ .

FIG. 2. Epiphyseal bone marrow of a guinea pig weighing 425 gm. at the beginning of the experiment. This animal had been injected with 2 cc. of cattle anterior pituitary gland extract daily for 4 days. Islands of pseudoxanthomatous cells are seen.  $\times 220$ .



I

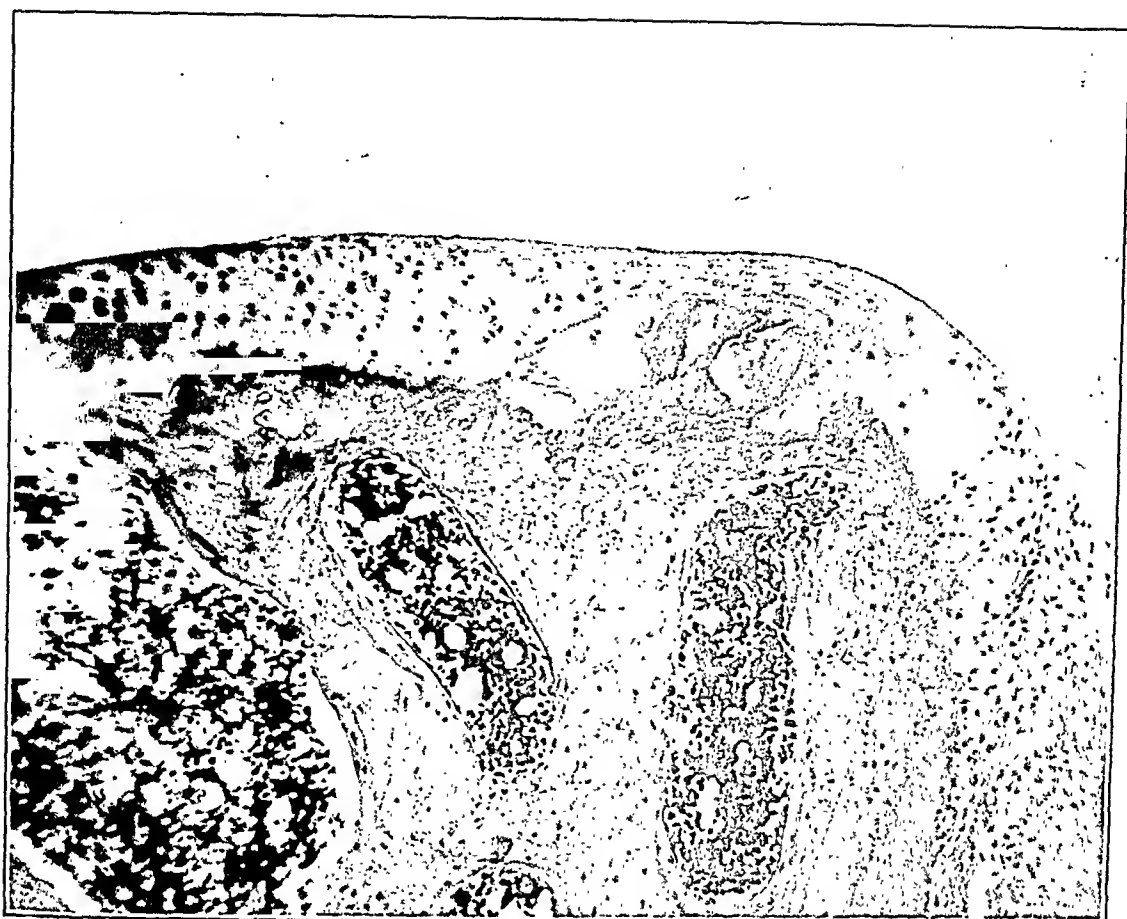


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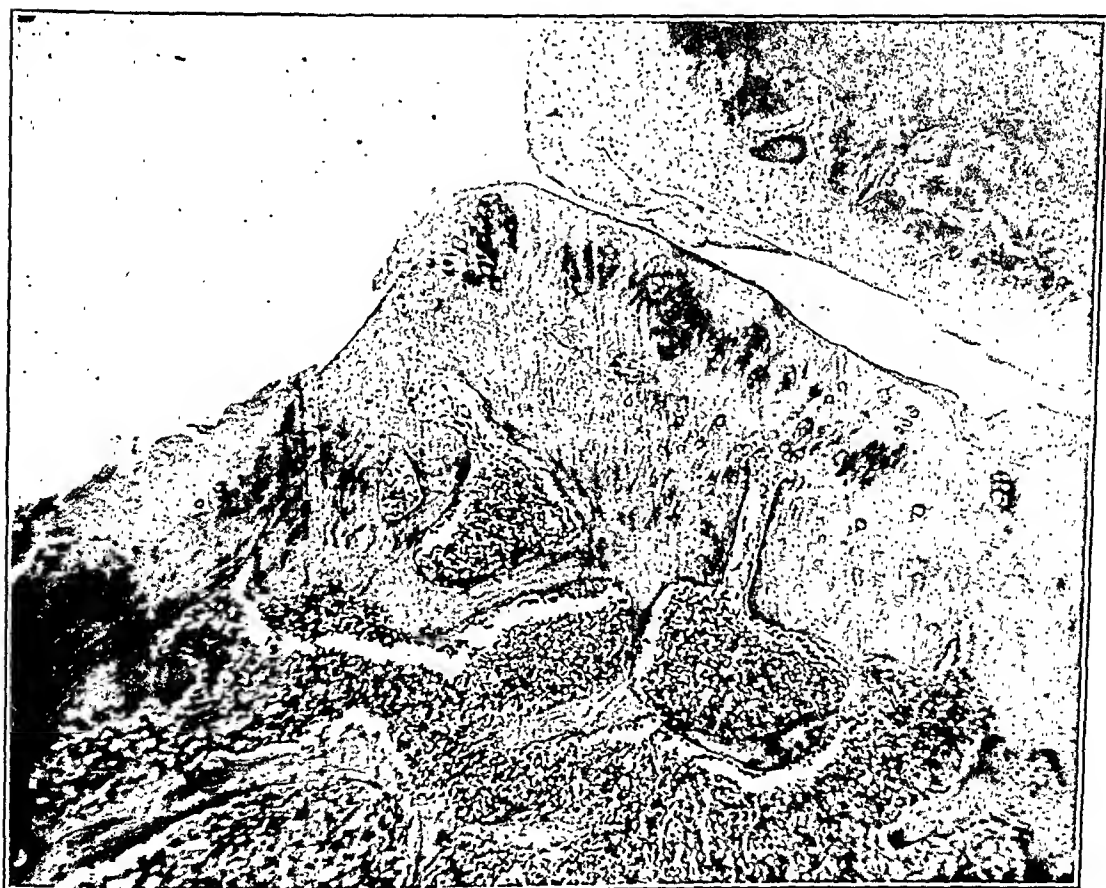
PLATE 91

FIG. 3. Surface of the head of the femur of a guinea pig weighing 750 gm. at the beginning of the experiment and which had received 4 cc. of the extract daily for 60 days. Some hyperplasia of the cartilage cells as well as localized but marked degeneration of the cartilaginous articular surface is seen.  $\times 150$ .

FIG. 4. Knee joint of the same guinea pig illustrated in Fig. 3. Ulceration of the articular cartilage with partial baring of the subchondral bone is seen. The persisting cartilage shows hypertrophy as well as areas of degeneration. Capillary loops from the bone marrow advance towards the surface of the joint.  $\times 150$ .



3



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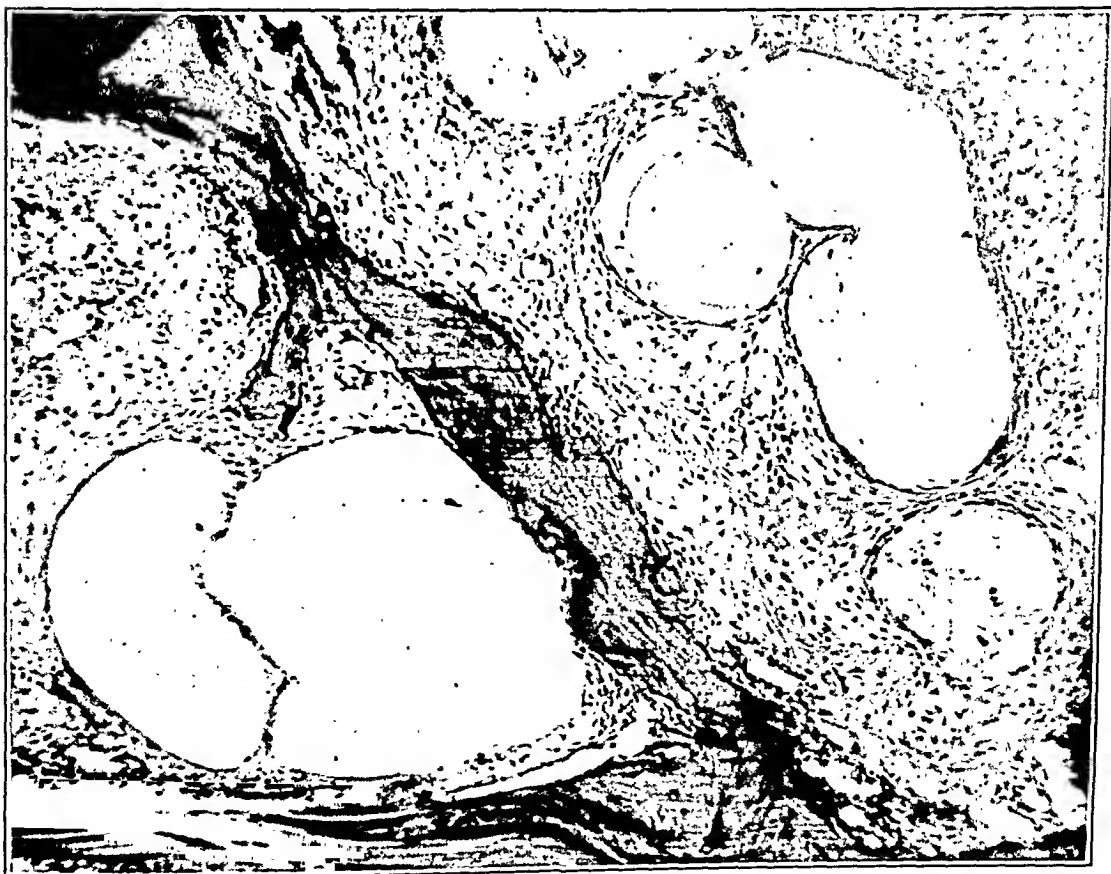
PLATE 92

FIG. 5. A different area from the joint shown in Fig. 4. Diffuse degeneration of hypertrophic cartilage and formation of villi on the free surface of the joint is present.  $\times 150$ .

FIG. 6. Epiphyseal bone marrow of a guinea pig weighing 760 gm. at the beginning of the experiment and which had been injected with 4 cc. of the extract daily for 6 days. A formation of cysts has taken place in the myxoid connective tissue.  $\times 150$ .



5

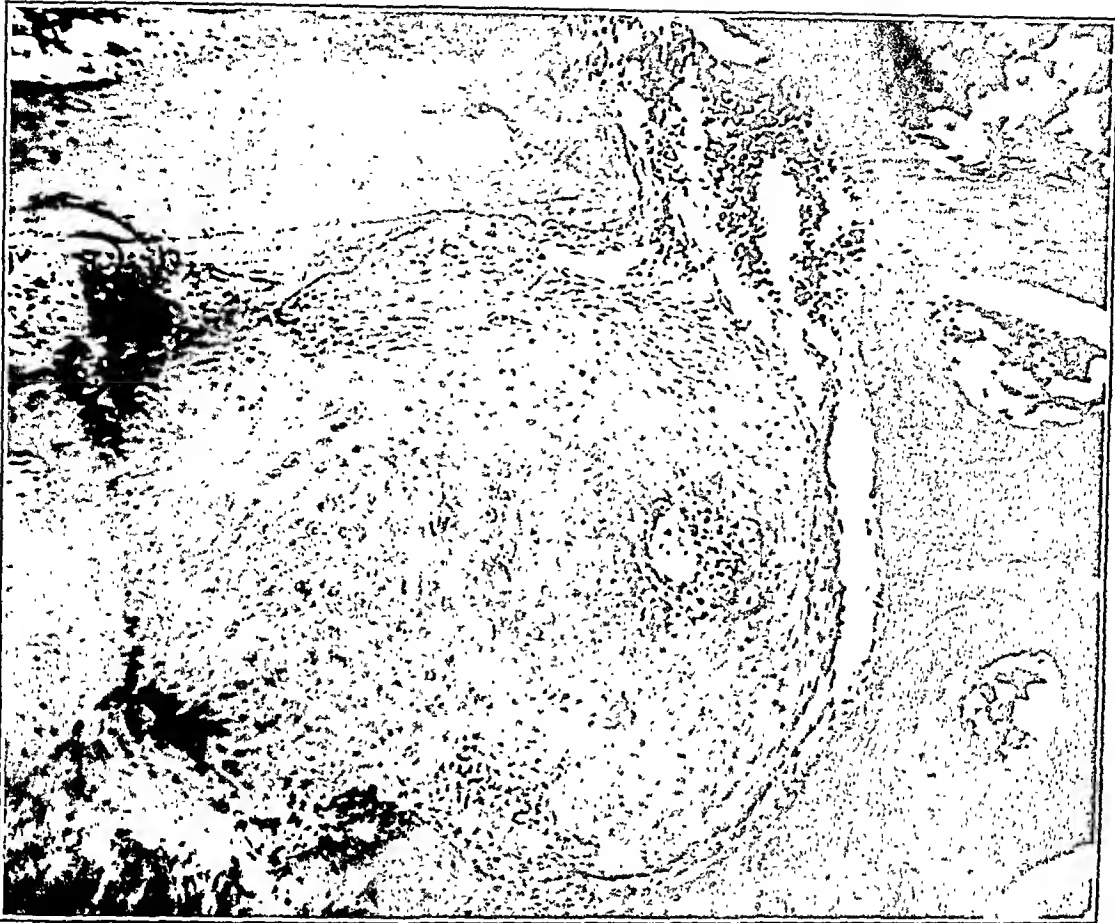


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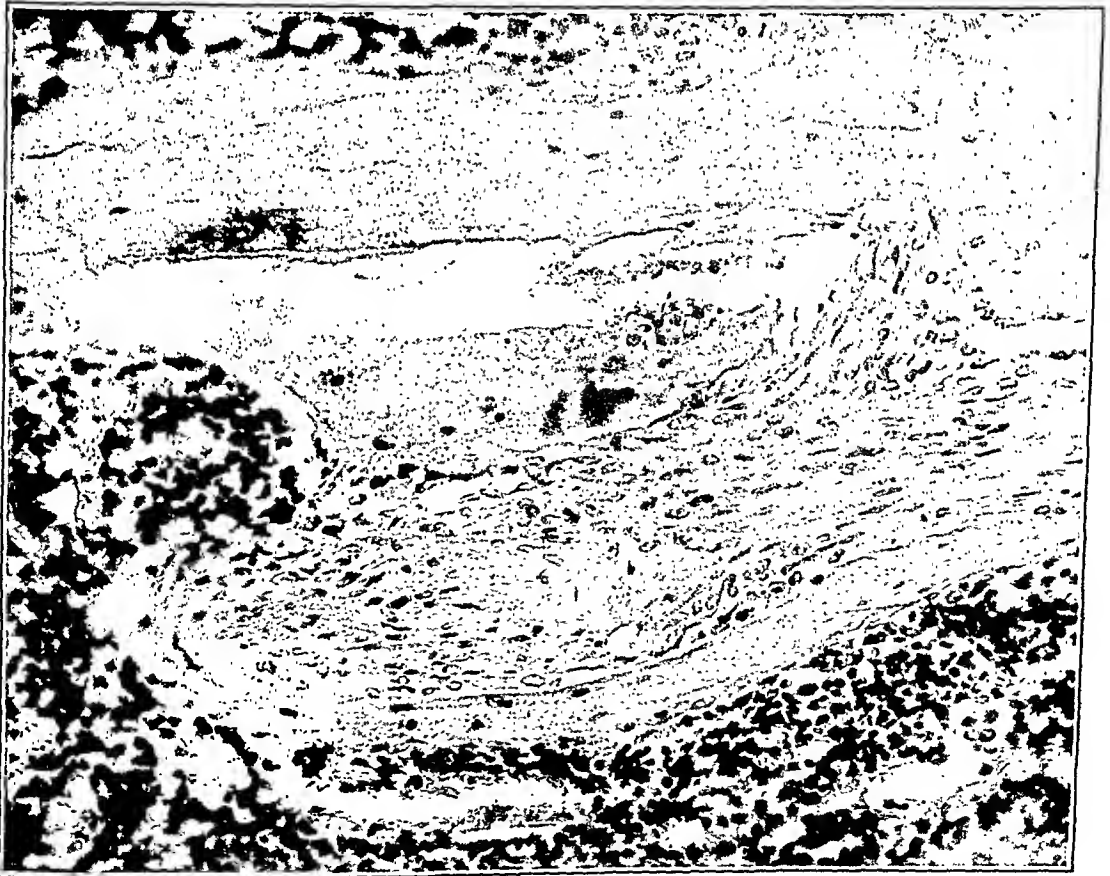
PLATE 93

FIG. 7. Epiphyseal bone marrow of a guinea pig weighing 875 gm. at the beginning of the experiment and which had been injected with 4 cc. of the extract daily for 14 days. Formation of cartilage in the bone marrow with liquefaction in the center is to be seen.  $\times 150$ .

FIG. 8. Epiphyseal bone marrow of the same guinea pig illustrated in Fig. 3. Resorption and substitution of trabeculae by fibrous tissue is present.  $\times 220$ .



7



8





# INTRACELLULAR BACILLI IN INTESTINAL AND MESENTERIC LESIONS OF TYPHOID FEVER \*

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In 1937 Goodpasture<sup>1</sup> reported the occurrence of small Gram-negative intracellular bacilli, judged to be *Eberthella typhi*, within the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum and in mesenteric lesions of early cases of typhoid fever. Larger Gram-negative bacilli were found concurrently in these lesions in necrotic macrophages. These observations were made on 5 cases of human typhoid fever which had come to autopsy early in the disease. It was concluded that *E. typhi* is capable of growing in both of these situations, and the inference was made that the young plasma cell is an essential cellular host in the typical human disease and serves as a nourishing and protecting medium, not only during the period of incubation but throughout the active course of typhoid fever. He was led to study the lesions of typhoid fever after the observation of small, intracytoplasmic colonies of bacilli in the entodermal epithelial cells of the chorio-allantois of chick embryos infected with *E. typhi*.<sup>2</sup>

It is the purpose of this paper to report a similar study of 6 additional cases of typhoid fever.†

Zenker-fixed paraffin sections stained in Wright's stain (60 drops to 100 cc. of distilled water) for about 4 hours, differentiated in absolute ethyl alcohol, cleared in xylol and mounted in cedar oil were used for study. With this method the intracytoplasmic bacillary forms are somewhat inconspicuous owing to the blue-staining properties of both the cytoplasm of the plasma cells and the bacteria, in conjunction with the relatively small size of the bacterial forms. The following staining method was therefore

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† Of the cases studied, 2 came from the Department of Pathology of Vanderbilt University. Material from 1 case was supplied by Dr. W. A. DeMonbreun from the Pathological Laboratory of the Nashville General Hospital. Sections from 3 cases were kindly sent by Dr. W. D. Forbus from the Pathology Department of Duke University Hospital.

devised whereby better contrast could be obtained between the bacteria and the cytoplasm of the cells.

1. Stain in Weigert's iron hematoxylin solution for 1 minute or less.
2. Wash in 50 per cent alcohol acidulated to 0.1 per cent hydrochloric acid.
3. Stain in Goodpasture's carbol-aniline-fuchsin solution for 1 minute.
4. Decolorize in a 5 per cent aqueous solution of acetic acid until the red color disappears or the sections remain a light pink.
5. Repeat steps 3 and 4 three times.
6. Wash in water.
7. Stain in a 0.01 per cent light green solution, acidulated to 0.2 per cent acetic acid, for about 1 minute or until the section has a light green hue. Overstaining is to be avoided.
8. Wash in water, differentiate in 95 per cent alcohol, dehydrate in absolute alcohol, clear in xylol and mount in balsam.

The nuclei are stained black, the nucleoli red, the cytoplasm a pale green, bacteria a brilliant red, red blood cells red and other structures green.

#### ABSTRACT OF CASES

CASE 1, V-38-III: A 56 year old negro was admitted to the Vanderbilt Hospital in a stuporous condition with a story of diarrhea and fever of 4 days duration. The blood culture was positive for *E. typhi*. The course of illness was one of profound toxemia and was steadily downhill. Death occurred on the 3rd hospital day.

Autopsy showed hyperplastic Peyer's patches and solitary follicles in the distal 36 inches of the ileum. The lesions were in an early ulcerative phase. There was a mesenteric lymphadenitis and focal necrosis of the liver, spleen and kidneys. The blood culture and culture of the intestinal lesions were positive for *E. typhi*.

Intracytoplasmic bacillary forms were demonstrated in the intestinal lesions without difficulty. Cells of this type were rarely found in sections of the mesenteric nodes.

CASE 2, G-38-127: A 22 year old negro was admitted to the Nashville General Hospital 13 days before death with a history of fever of 4 days duration. The Widal test showed agglutination at 1:640 and the stool culture yielded *E. typhi*.

Autopsy revealed classical, hyperplastic lymphoid lesions in the distal 12 inches of the ileum and the proximal 24 inches of the colon. The appendix was inflamed and the mesenteric nodes were swollen. Microscopic studies revealed classical, pre-ulcerative, early necrotic, typhoid intestinal lesions, mesenteric lymphadenitis, and focal areas of necrosis in the spleen, liver, pancreas and adrenal. A blood culture at autopsy yielded a pure culture of *E. typhi*.

Intracytoplasmic bacillary forms were relatively numerous in the plasma cells in the involved Peyer's patches. They were demonstrated in the colon and in the mesenteric nodes. None was found in the appendix.

CASE 3, Duke No. 173: A 20 year old negro was admitted to the Duke University Hospital 5 days before death with a history of headache for 15 days and fever for 8 days. The blood culture was positive for *E. typhi*.

Autopsy revealed early ulceration of Peyer's patches and hyperplastic follicles in the proximal colon. There was a mesenteric lymphadenitis, an inflamed appendix and focal areas of necrosis in the spleen and liver.

Intracytoplasmic bacillary forms were observed in plasma cells in sections of the involved areas of the ileum and colon. The mesenteric nodes were not studied and no bacterial forms were found in the appendix.

CASE 4, Duke No. 257: A 15 months old white boy was admitted to the Duke University Hospital 9 days before death with a history of fever of 2 weeks duration. The blood culture was positive for *E. typhi* on admission and at autopsy.

Ulcerative lesions were present, but not marked, in the Peyer's patches and in the colon. Microscopically the lesions were in the ulcerative stage. The cellular response was atypical in that there were many more large mononuclear cells and fewer plasma cells than usual. There was a marked mesenteric lymphadenitis.

Intracytoplasmic bacillary forms were demonstrated infrequently in occasional plasma cells in a section of a Peyer's patch. None was found in other sections.

CASE 5, Duke No. 298: An 8 year old negro boy ill for 2 weeks before admission to the Duke University Hospital died on his 2nd hospital day.

At autopsy ulcerated lesions were present in the cecum, ascending colon and Peyer's patches. Microscopically the hyperplastic and ulcerative lesions in the intestines showed a somewhat atypical cellular response. In some areas there were large collections of polymorphonuclear leukocytes. There was a marked mesenteric lymphadenitis.

Plasma cells in a section of a Peyer's patch showed infrequent intracytoplasmic bacillary forms. None was found in the colon. Typical intracytoplasmic colonies were observed in large plasma cells in a section of a mesenteric node.

A 6th case, in which no intracytoplasmic bacillary forms were found, may be compared with the above group.

CASE 6, V-37-144: A 15 year old girl was admitted to the Vanderbilt Hospital in a semistuporous condition with a history of fever of 2 weeks duration. On her 4th hospital day a perforation was suspected and an exploratory operation was performed, during which two perforated ulcers in the lower ileum were closed. The course of illness was downhill until she died on the 7th hospital day.

Autopsy showed a diffuse peritonitis, deeply ulcerated Peyer's patches in the ileum and ulcerated lesions in the cecum and ascending colon. *E. typhi* was isolated from the bile but was not recovered from cultures of the blood stream or from the ulcers. Microscopically the lesions were necrotic and deeply ulcerated.

#### DISCUSSION

This study confirms the previous observations of Goodpasture that the intracytoplasmic bacillary forms within plasma cells of intestinal and mesenteric lymphoid lesions of typhoid fever are most numerous in early cases. They are found in areas where necrosis has not yet taken place or is limited in extent and where cellular hyperplasia and infiltration are marked. They are most frequently found in hyperplastic areas over which the mucosa is still intact.

The observations here recorded supplement the previous report in that intracytoplasmic forms were demonstrated in colonic lesions as well as in lesions in the ileum and in involved mesenteric nodes. They were also demonstrated in childhood lesions of

typhoid where the cellular reaction consists more of a large mononuclear infiltration with an associated polymorphonuclear invasion.

In one instance also the small bacillary forms were found within the cytoplasm of a lymphoblast or plasma cell in the process of mitosis. This observation indicates that the bacilli may cause little or no injury to the host cell, and that the latter, stimulated to mitosis, might give rise to two infected cells without reinfection. The frequent observation of two or more infected plasma cells in close proximity to each other suggests the possibility also of reproduction of infected cells.

The intracytoplasmic colonies of bacillary forms, as seen in plasma cells, are surrounded by a narrow clear zone. In some cells there is only a single colony composed of a few bacteria while in others there are as many as six colonies. Some colonies contain a large number of bacteria, perhaps 100 or more. In occasional cells containing large colonies the vacuoles in which they lie appear to have ruptured and the bacilli appear to be exuding into the adjacent tissue. The bacillary forms are Gram-negative and all indications are that they are small forms of *E. typhi*.

The apparent regularity in the occurrence of intracellular bacterial forms within young plasma cells in the intestinal and mesenteric lesions in early cases of typhoid fever indicates that they are an essential component of these classical early lesions and that they are of importance in the pathogenesis of this disease.

#### SUMMARY AND CONCLUSION

1. Gram-negative, intracytoplasmic bacillary forms, judged to be *E. typhi*, have been found in the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum, colon and mesenteric lymph nodes in 5 cases of early typhoid fever.
2. A method for staining intracellular bacteria is described.
3. It is concluded that the presence of these bacillary forms within the plasma cell is an essential part of the early, classical, intestinal and mesenteric lesions of typhoid fever.

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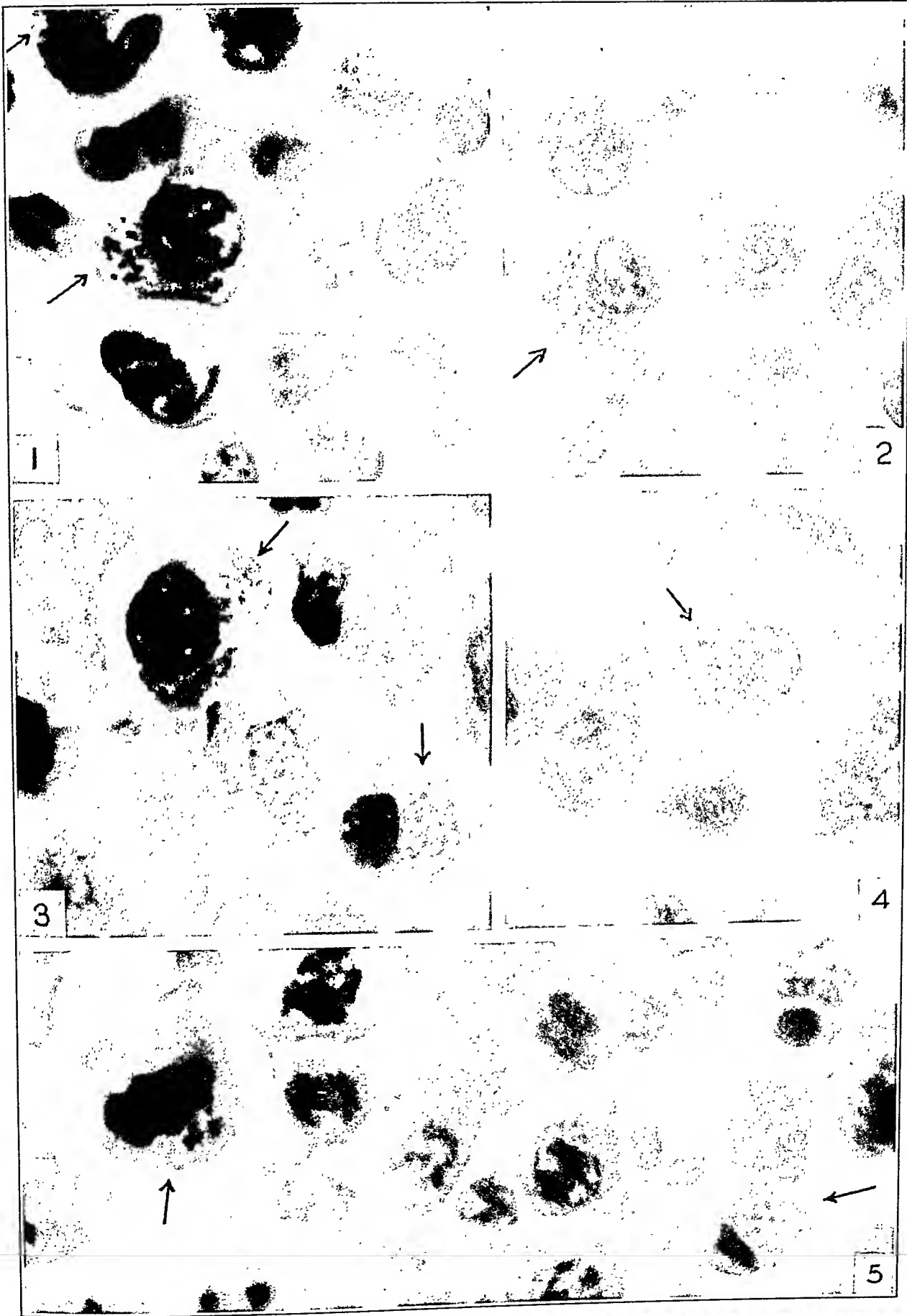
DESCRIPTION OF PLATE

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## PLATE 94

FIGS. 1-4. Microphotographs showing young plasma cells of a persisting follicle in a Peyer's patch. The arrows point to groups of intracellular bacilli. Note the encapsulating material about the bacilli. Basic fuchsin-light green stain.  $\times 2500$ .

FIG. 5. The cell on the left containing intracellular bacilli is undergoing mitosis.  $\times 2500$ .







## CARCINOMA OF THE LUNG \*

### AN ANALYSIS OF SEVENTY-FOUR AUTOPSIES

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Two previous communications from this laboratory have dealt respectively with 40 cases of primary carcinoma of the biliary system<sup>1</sup> and 40 cases of primary carcinoma of the pancreas.<sup>2</sup> The present study deals with 74 cases of primary carcinoma of the lung. As in the previous communications, this report is principally concerned with the site and structure of the primary growth and with its spread locally and to distant parts. The clinical manifestations, their duration, and the immediate causes of death are also briefly considered.

*Race, Sex and Age:* The 74 cases of carcinoma of the lung were encountered in 6623 autopsies on individuals over 1 year of age performed between Jan. 1, 1931, and June 30, 1938 by the staff of the Department of Pathology of the Charity Hospital of Louisiana at New Orleans. Forty-seven patients were white (42 male, 5 female) and 27 negro (26 male and 1 female). The youngest patient was 21 and the oldest 75 years of age. One died in the 3rd, 4 in the 4th, 13 in the 5th, 33 in the 6th, 19 in the 7th, and 4 in the 8th decade of life (Tables I, II and III).

*Site and Structure of Neoplasms:* The main growth was located in the right lung in 38 cases and in the left lung in 33. In the 3 remaining cases the site could not be determined. In 35 cases the primary growth was located in one bronchus or the other, 6 in this group being located near or at the bifurcation of the trachea (Fig. 1). Twenty-eight growths were located in a branch of a bronchus (Fig. 2).

The diameter of the mass forming the primary growth varied from 2 to 15 cm. Ulceration of the bronchial mucosa was frequently observed, together with involvement of the bronchial wall and of the underlying pulmonic tissue. The affected bronchus was usually identified near the periphery of the growth and not

\* Received for publication March 11, 1939.

TABLE I  
Data on Patients with Squamous Cell Carcinoma

Number of case	Age	Sex and race	Site *		Spread		Clinical manifestations	Duration of illness mos.	Cause of death
			Right	Left	Regional	Distant			
S-1 33-1362	yrs. 33	M W	B		Lymph nodes	Lymph nodes, pancreas	Pain, cough, dyspnea	3	Carcinoma
S-2 35-806	42	M W		B	Lymph nodes		Cough	6	Carcinoma
S-3 37-1021	42	M C		B U	Ribs	Lymph nodes	Pain, dyspnea, hemoptysis	9	Carcinoma
S-4 32-540	49	M C	B U	B U	Lymph nodes	Lymph nodes	Cough, dysphagia	1	Carcinoma
S-5 32-277	50	M W			Lymph nodes	Lymph nodes	Pain, cough, loss of weight	5	Abscess of lung
S-6 32-1047	50	M C	Lung	Lung	Lymph nodes, pericardium	Liver, adrenals, skeleton	Pain, loss of weight	2	Carcinoma
S-7 38-233	50	M C		B	Lymph nodes	Lymph nodes	Hemoptysis	1	Carcinoma
S-8 31-371	51	M W		B U	Lymph nodes	Liver, adrenals, pancreas			Abscess of lung
S-9 34-309	51	M C	B		Lymph nodes		Pain, cough, dyspnea, hemoptysis	3	Carcinoma
S-10 36-1390	51	M W		Lung	Pleura		Pain	3	Carcinoma
S-11 33-448	53	M W		B U	Lymph nodes		Pain, dyspnea	2	Carcinoma
S-12 34-915	53	M C	B L		Lymph nodes		Pain	9	Abscess of lung
S-13 33-83	54	M W	B			Liver, pancreas	Cough, dyspnea, hemoptysis	6	Abscess of lung
S-14 33-275	54	M W	B L				Cough	5	Abscess of lung
S-15 37-526	54	M C	Lung				Cough, loss of weight	5	Carcinoma
S-16 36-420	55	M W		B	Pleura	Kidney	Pain, cough, dyspnea	5	Carcinoma
S-17 38-176	55	M C	B L			Lymph nodes	Dyspnea, dysphagia	4	Carcinoma

\* B = bronchus; U = upper; L = lower; M = middle.

S-18 37-1037	57	M W		B	Lymph nodes, pleura	Liver, skeleton, skin	Pain, cough, loss of weight Cough Dyspnea	1	Carcinoma
S-19 33-563	58	M W	B		Lymph nodes				Carcinoma
S-20 37-885	58	M C	B L					1	Obstruction vena cava inferior
S-21 38-111	58	M C	B		Lymph nodes		Pain, cough, hemoptysis	1	Carcinoma
S-22 32-124	59	M W		B L	Lymph nodes, pleura		Pain, cough, dyspnea	18	Abscess of lung
S-23 33-1277	59	M C			Lymph nodes, pericardium	Intestine Liver	Cough, loss of weight, hemop- tysis	12	Abscess of lung
S-24 38-114	59	M C	B U	B				5	Carcinoma
S-25 36-1512	60	M W		B	Lymph nodes	Skeleton	Cough, loss of weight, hemop- tysis	6	Carcinoma
S-26 32-967	61	M W		B L	Lymph nodes, pericardium		Pain, dysphagia	5	Carcinoma
S-27 36-1083	61	M W			Lymph nodes		Pain	4	Carcinoma
S-28 37-333	62	M W	Lung	B	Lymph nodes, pleura, peri- cardium		Pain, cough, loss of weight, hemop- tysis	12	Abscess of lung
S-29 38-55	63	M W	Lung			Liver	Pain, dyspnea, loss of weight	7	Carcinoma
S-30 37-840	63	F W		B U	Lymph nodes, pericardium	Kidneys	Pain	5	Carcinoma
S-31 35-1162	64	M W	B U		Pleura			4	Carcinoma
S-32 37-1237	64	M C	B		Lymph nodes	Lymph nodes	Pain, cough, loss of weight	1	Carcinoma
S-33 38-376	64	M W		B	Lymph nodes	Liver, kidneys	Pain, dyspnea Dyspnea, hemop- tysis	7	Carcinoma
S-34 38-77	65	M W		B			Loss of weight	3	Carcinoma
S-35 36-342	68	M C		B			Dyspnea, loss of weight	6	Carcinoma
S-36 33-1082	73	M C		B			Cough		Abscess of lung
S-37 37-1031	74	F W	Lung	Lung					Thrombosis, coro- nary artery

TABLE II  
Data on Patients with Reserve Cell Carcinoma

Number of case	Age yrs.	Sex and race	Site *		Spread		Clinical manifestations	Duration of illness mos.	Cause of death
			Right	Left	Regional	Distant			
R-1 33-59	21	M C		B U	Lymph nodes, pleura	Skeleton	Paralysis	1	Carcinoma, transverse myelitis
R-2 37-54	36	M W	Lung	B	Lymph nodes	Kidneys	Pain, dyspnea	4	Carcinoma
R-3 37-955	40	M C			Lymph nodes		Pain, cough, loss of weight	1	Pneumonia, diffuse
R-4 32-354	46	M W		B U			Pain	4	Abscess of lung
R-5 36-304	47	M C	B	B	Lymph nodes	Liver	Cough	4	Abscess of lung
R-6 32-112	48	M W			Lymph nodes	Kidneys, skeleton, meninges	Dyspnea, loss of weight	3	Carcinoma
R-7 33-2	51	F C				Liver	Pain, cough	1	Carcinoma
R-8 33-306	51	M C	Lung	Lung	Pleura		Loss of weight, dysphagia	6	Carcinoma
R-9 37-1043	54	M W		B	Lymph nodes		Dyspnea	1	Lobectomy
R-10 35-180	56	M W	B L		Pleura	Lymph nodes, liver, pancreas	Pain		Carcinoma
R-11 31-99	57	M W	B U		Lymph nodes	Liver	Pain, cough		Carcinoma
R-12 36-212	57	M W		B			Cough, loss of weight, hemoptysis	3	Pneumonia, diffuse

\* B = bronchus; U = upper; L = lower; M = middle.

R-13 34-785	58	M W	B		Lymph nodes		Pain, cough Cough, dyspnea	2	Abscess of lung
R-14 37-790	58	M W	B					6	Constriction vena cava superior Carcinoma
R-15 36-204	59	M W	B M		Lymph nodes	Liver, adrenals, pancreas Liver	Pain, hemoptysis	7	Carcinoma
R-16 35-1062	60	M C	B		Lymph nodes, pleura				Carcinoma
R-17 34-907	61	M W		Lung	Lymph nodes	Liver, pancreas	Pain	1	Carcinoma
R-18 34-933	68	M C		B	Lymph nodes	Lymph nodes, liver	Dysphagia	3	Carcinoma
R-19 36-490	68	M W	B L		Lymph nodes				Abscess of lung
R-20 34-905	72	M W	B L		Lymph nodes, pleura	Liver	Pain, cough, loss of weight	6	Carcinoma
R-21 36-1458	75	M W		B	Lymph nodes		Pain, cough	24	Carcinoma

TABLE III  
Data on Patients with Columnar Cell Carcinoma

Number of case	Age yrs.	Sex and race	Site *		Spread		Clinical manifestations	Duration of illness mos.	Cause of death
			Right	Left	Regional	Distant			
C-1 31-85	39	FW	B U		Lymph nodes	Lymph nodes	Pain	1	Constriction vena cava superior Carcinoma
C-2 34-163	48	MC		Lung	Lymph nodes, diaphragm Lymph nodes	Lymph nodes, liver, pancreas Lymph nodes	Pain, dyspnea	3	Abscess of lung
C-3 34-747	48	MW	B		Lymph nodes		Pain, loss of weight, hemoptysis Cough, hemoptysis	3	Carcinoma
C-4 36-346	49	MW	B		Lymph nodes		Pain, cough Pain, dyspnea	7	Abscess of lung
C-5 35-195	50	MW	B		Lymph nodes		Pain, cough	8	Carcinoma
C-6 36-96	52	MW	B U		Lymph nodes, diaphragm		Pain, dyspnea	9	Lobectomy
C-7 37-439	52	MC	B		Lymph nodes, pleura		Pain, dyspnea	4	Carcinoma
C-8 38-434	52	MC	B L		Lymph nodes, pleura	Liver, adrenals, pancreas	Pain, dyspnea, hemoptysis	6	Carcinoma
C-9 34-597	54	FW	B		Lymph nodes, pleura	Liver, adrenals, pancreas, spleen	Cough, dysphagia Pain, cough	12	Carcinoma
C-10 35-1060	54	MW	B U		Lymph nodes, ribs	Lymph nodes, adrenal	Cough, dysphagia	5	Carcinoma
C-11 36-160	61	MW	B		Lymph nodes	Adrenals, skeleton	Cough	1	Carcinoma
C-12 38-222	62	MC	B		Diaphragm	Lymph nodes, skeleton	Pain, cough, loss of weight	5	Carcinoma
C-13 34-1295	64	MC	B		Pleura, ribs	Adrenals, lymph nodes	Cough, dyspnea, loss of weight	3	Carcinoma
C-14 35-442	65	MW	B L		Lymph nodes, pleura	Kidneys	Cough, hemoptysis	5	Carcinoma
C-15 34-952	67	FW	B L		Lymph nodes			5	Carcinoma
C-16 32-859	68	MW	B		Lymph nodes			5	Carcinoma

\* B = bronchus; U = upper; L = lower; M = middle.

in the center (Fig. 3). The primary growths and their metastatic foci varied in gross appearance, the variations seeming to depend principally on the rate of growth, the amount and character of the stroma, and such secondary changes as hemorrhage and necrosis, rather than upon the actual cellular structure of the tumor parenchyma. It was therefore impossible to set up definite criteria by which on gross examination the microscopic structure of the carcinoma could be predicted with any degree of certainty.

Following the histogenetic classification previously outlined by one of us (B. H.<sup>3</sup>), the neoplasms were divided into three groups on the basis of their microscopic structure — squamous cell, reserve cell and columnar cell carcinoma. Thirty-seven of the 74 neoplasms were squamous cell, 21 reserve cell and 16 columnar cell carcinoma.

The squamous cell carcinoma was usually composed of nests or sheets of tumor cells arranged more or less concentrically to form epithelial pearls (Fig. 4). In some growths the cells toward the center of the cell sheets disclosed varying degrees of keratinization (Fig. 5), or were transformed into scales or into cell debris (Fig. 6).

The reserve cell carcinoma was composed of sheets or solid masses of tumor cells, which formed no particular structure (Fig. 7). Usually the cytoplasm was scant and the cell borders hardly discernible. The nuclei of the cells were fairly uniform, ovate or elongated, and stained deeply. In some growths the cells seemed to be arranged in whorls (Fig. 8), in others there was a palisade arrangement of the peripheral cells (Fig. 9).

The columnar cell carcinoma was usually composed of columnar or cuboidal cells, and of solid masses of undifferentiated tumor cells. The columnar or cuboidal cells formed acinar or tubular structures which simulated in a haphazard way the normal epithelial structures of the air passages (Figs. 10 and 11). In some growths these cells were mounted on connective tissue stalks in a papillary arrangement (Fig. 12). Columnar cells forming acinar or tubular structures were occasionally observed in predominantly squamous cell growths.

In all three types of carcinoma of the lung there was a wide variation in the number of nuclei in mitosis. The amount and



density of the stroma, the degree of infiltration with lymphocytes and plasma cells, and the extent of areas of necrosis and hemorrhage also varied in the individual growths, as well as in different fields of the same growth.

*Manner of Spread:* Local extension with involvement of the regional lymph nodes occurred in 65 of the 74 cases (87.8 per cent), and extensive distant metastases in 41 (55.4 per cent). Metastatic foci were encountered in the liver 19 times, in the pancreas and suprarenal glands 8 times each, and in the kidneys and in the skeleton 7 times each.

*Clinical Course:* Thirty-seven of the 74 patients complained of pain in the chest, neck or epigastrium. Thirty-five complained of cough, with or without expectoration, 21 of dyspnea, 19 of loss of weight, 15 of hemoptysis, and 6 of dysphagia.

In the 65 cases in which information was available, the illness had lasted from 1 to 24 months, with an average duration of 5 months. The newgrowth was the principal lesion and the immediate or contributory cause of death in 71 patients.

#### COMMENT

The increasing importance of carcinoma of the lung as a clinical problem is made clear in such recent publications as those of Hruby and Sweany,<sup>4</sup> Tuttle and Womack,<sup>5</sup> Rabin and Neuhof,<sup>6</sup> Jackson and Konzelmann,<sup>7</sup> Graham,<sup>8</sup> Kennaway and Kennaway,<sup>9</sup> Husted and Biilmann,<sup>10</sup> Simons,<sup>11</sup> Mattick and Burke,<sup>12</sup> Edwards,<sup>13</sup> Klotz,<sup>14</sup> Matz,<sup>15</sup> Howes and Schenck,<sup>16</sup> Hochberg and Lederer,<sup>17</sup> Tod,<sup>18</sup> and Ochsner and DeBakey.<sup>19</sup> New opportunities for the analysis and clarification of some of its morphological problems are presented in the large series of cases studied at autopsy and reported in such recent communications as those of Geschickter and Denison,<sup>20</sup> Neely,<sup>21</sup> Olson,<sup>22</sup> Jaffé,<sup>23</sup> Lindberg,<sup>24</sup> Samson,<sup>25</sup> Rice,<sup>26</sup> Frissell and Knox,<sup>27</sup> Brines and Kenning,<sup>28</sup> Bauer,<sup>29</sup> and Koletsky.<sup>30</sup>

There is an apparently wide variance in the conceptions of individual authors as to the histogenesis and structure of carcinoma of the lung. The available data have contributed materially to our knowledge, but no uniformity has as yet been attained in classifying these growths. The difficulty may be superficial, however, rather than essential. In our opinion a classification on a

histogenetic basis, combined with a nomenclature derived from the cell making up the growth, rather than from the structure which the cell forms, will go far toward simplification of the problem.

All carcinomas primary in the lung, it now seems clear, can be classified into one of three groups — squamous cell, reserve cell and columnar cell. The occasional overlapping of groups is not unexpected, since all carcinomas of the lung, we believe, are derived from a common ancestor cell, the reserve cell (Fried<sup>31</sup>).

### SUMMARY

1. Seventy-four cases of primary carcinoma of the lung were encountered in 6623 autopsies on individuals over 1 year of age. Males and females were represented in the proportion 11:1. The age range was from 21 to 75 years. The average duration of illness was 5 months. Thirteen patients died in the 5th, 33 in the 6th, and 19 in the 7th decade of life.

2. In almost half of the cases the primary growth was located in one bronchus or the other.

3. Thirty-seven of the 74 cases were squamous cell, 21 were reserve cell, and 16 were columnar cell carcinoma.

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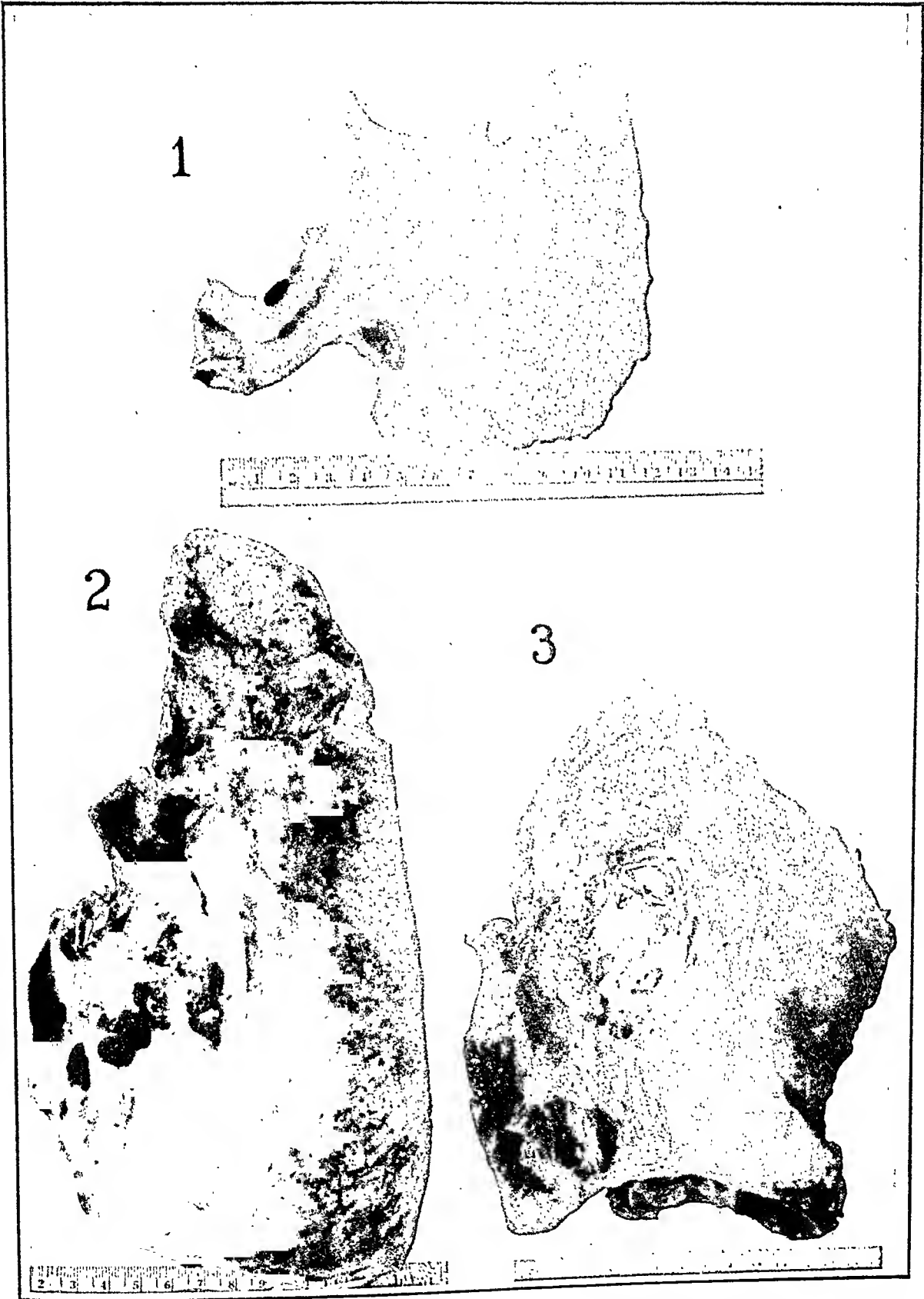
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## DESCRIPTION OF PLATES

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### PLATE 95

- FIG. 1. Primary growth in the right bronchus at the bifurcation of the trachea. The neoplastic infiltration extends through the entire thickness of the wall (R 13).
- FIG. 2. Primary growth in the hilus of the right lung in the wall of the bronchial branch to the lower lobe (R 20).
- FIG. 3. Primary growth in the hilus of the left lung, arising from the bronchial wall. The bronchus lies along the anterior margin of the growth and not in the center (S 34).



D'Aunoy, Pearson and Halpert

Carcinoma of Lung

PLATE 96

- FIG. 4. Squamous cell carcinoma composed of nests or sheets of tumor cells arranged more or less concentrically to form epithelial pearls (S 14).
- FIG. 5. Squamous cell carcinoma. The cells toward the center of the cell sheets disclose varying degrees of keratinization (S 24).
- FIG. 6. Squamous cell carcinoma. The cells toward the center of the cell sheets disclose varying degrees of keratinization and a cell débris (S 13).





PLATE 97

- FIG. 7. Reserve cell carcinoma composed of sheets or solid masses of tumor cells forming no particular structure. The cytoplasm is scant and the cell borders hardly discernible. The cell nuclei are fairly uniform, ovate or elongated, and stain deeply (R 6).
- FIG. 8. Reserve cell carcinoma. The cells seem to have a whorl-like arrangement (R 19).
- FIG. 9. Reserve cell carcinoma. A palisade arrangement of the peripheral cells is seen (R 20).

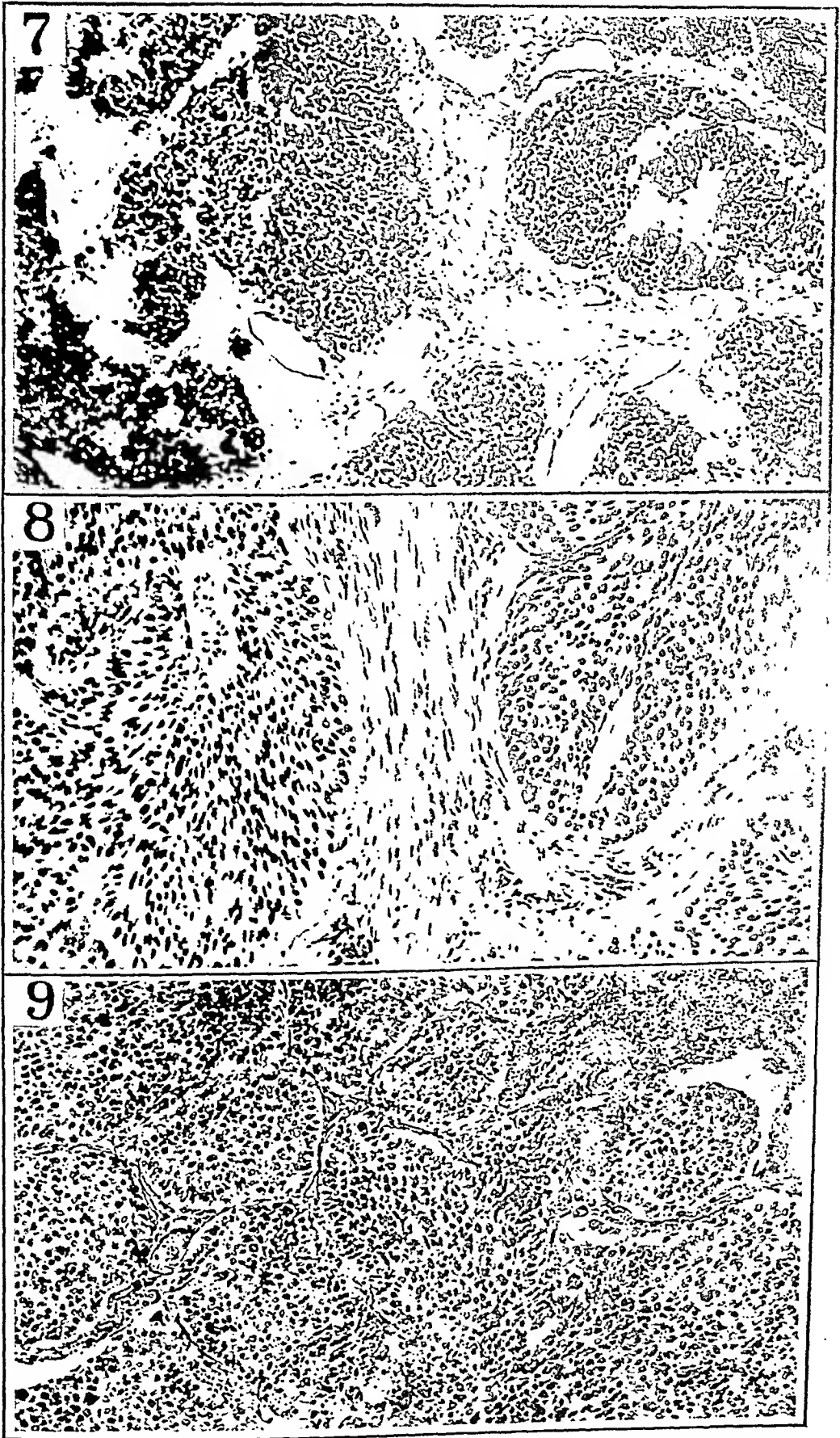
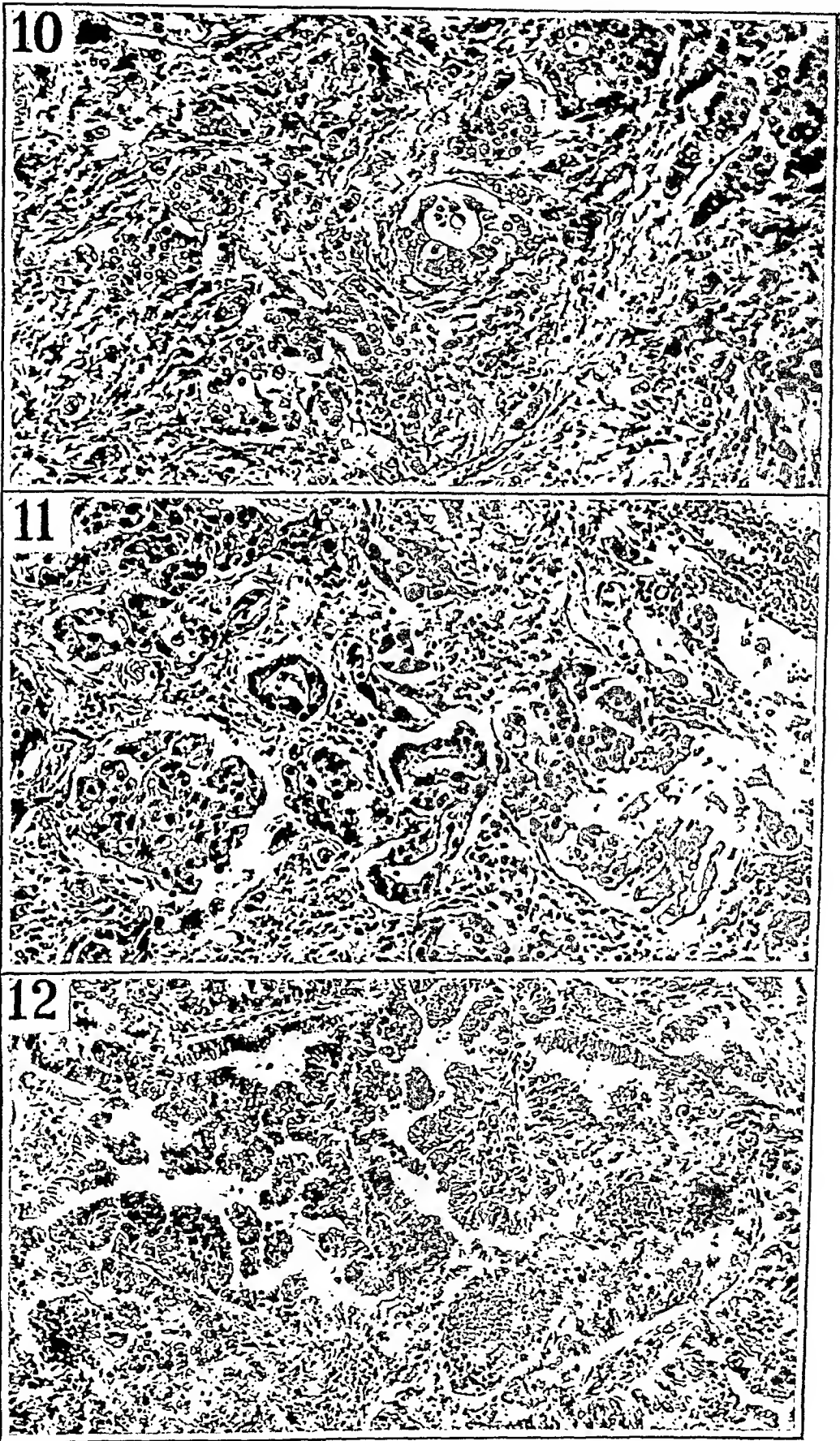
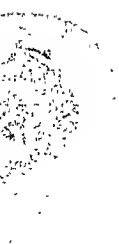


PLATE 98

FIGS. 10 and 11. Columnar cell carcinoma. The columnar or cuboidal cells, in acinar or tubular arrangement, simulate in a haphazard way normal epithelial structures of the air passages (C 12 and C 2).

FIG. 12. Columnar cell carcinoma. Tall columnar cells are mounted on connective tissue stalks in a papillary arrangement (C 11).





SCIENTIFIC PROCEEDINGS OF THE  
THIRTY-NINTH ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS  
HELD AT RICHMOND, VIRGINIA  
APRIL 6TH AND 7TH, 1939



BUSINESS MEETING  
OF  
THE AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS

Held in the Library, Medical College of Virginia,  
Richmond, Virginia

April 6, 1939

VICE-PRESIDENT WELLER PRESIDING

On nomination of the Council the Association voted to instruct the retiring member of the Council, Dr. William Boyd, to cast the ballot for election of the following officers:

<i>President</i>	CARL V. WELLER
<i>Vice-President</i>	STANHOPE BAYNE-JONES
<i>Treasurer</i>	FRANK B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	WILEY D. FORBUS
<i>Assistant Treasurer</i>	FREDERIC PARKER, JR.
<i>Assistant Secretary</i>	FRANCIS BAYLESS

On nomination of the Council the Association voted to elect Dr. Alan R. Moritz to fill the unexpired term of Dr. Earl B. McKinley. This term expires in 1942.

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Voted to elect the following new members:

Archie H. Baggenstoss	John W. Hall
Orville T. Bailey	Francis F. Harrison
Harvey P. Barret	George M. Hass
Oscar O. Christianson	Milton Helpern
Dale Rex Coman	Arthur T. Hertig
Warren C. Corwin	Russell L. Holman
Oran I. Cutler	Cornelius A. Hospers
Ralph L. Ferguson	John S. Howe
Harold Fink	George W. Jones



Lester S. King  
Kurt E. Landé  
Edwin H. Lennette  
Victor Levine  
Fritz Levy  
Averill A. Liebow  
Leon S. Lippincott  
David G. Mason  
William L. McNamara  
Arthur A. Nelson  
Robert J. Parsons  
S. Milton Rabson

Alex B. Ragins  
Jacob M. Ravid  
Philipp Rezek  
Paul S. Rhoads  
Walter Schiller  
Joseph Schleifstein  
Hans Smetana  
Edith E. Sproul  
Joseph Victor  
Emory D. Warner  
William B. Wartman  
Jarrett E. Williams

Voted to accept with regret the resignations of Drs. M. F. Boyd, A. G. Ellis, F. R. Sabin, E. McD. Stanton, R. P. Strong and W. H. Watters.

Voted to record with deep regret the deaths of Drs. W. H. Chase, W. C. Johnson, H. A. McCordock, E. B. McKinley, and M. J. Sittenfield.

The Secretary announced that the next meeting of the Association will be held at the University of Pittsburgh, Pittsburgh, Pennsylvania, March 21 and 22, 1940.

The Secretary announced that the Symposium for next year will be on the subject of the Pathology of Vitamin Deficiencies and that there will be no formally selected referee.

The Secretary read the following resolution, adopted by the Council:

Since the agreement between the American Chemical Society, the American Medical Association and the American Association of Pathologists and Bacteriologists was consummated in 1924, the policy of the American Association of Pathologists and Bacteriologists has changed in the direction of restricting its activities to the "advancement of the knowledge of disease." For this reason the American Association of Pathologists and Bacteriologists, without prejudice, withdraws from the agreement.

The Secretary announced that in the future contributions to the program, including illustrative material, shall be presented within a limit of 10 minutes, except in the case of invited guests.

Voted to authorize the publication in the Scientific Proceedings

of the Association abstracts of papers listed in the program as "Read by Title."

The Secretary then proposed to the Vice-President that the members and guests of the Association stand for a moment's silent tribute to the memory of Dr. Earl B. McKinley.

The scientific session proceeded as in the following program.



## AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

PATHOGENETIC STUDIES OF TUBERCULOUS LESIONS IN ADULTS. Kornel L. Terplan, Buffalo, N. Y.

*Abstract.* Among 267 adults between 19 and 80 years of age recent primary tuberculosis was found in 33, two typical tuberculous complexes (Ranke) of different ages (one healed, the other recent) in 22, and a recent caseated complex in the presence of a healed primary focus without corresponding lesions in the lymph nodes was found in 7 cases. In addition a primary focus without gross or microscopic evidence of tuberculosis in the regional lymph nodes was present in 20 cases. Recent focal tuberculous lesions, as incidental findings, mostly in one subapical area, in the presence of a healed stony complex, were found in 17 cases. In none of these were hematogenous lesions observed. In 13 cases with a recent caseated complex as an incidental finding one or more additional caseated foci in subapical fields or in lower lobes were seen which had the same histological structure as the primary focus. There was no evidence of hematogenous seeding in these cases. Chronic pulmonary tuberculosis with intrabronchial spread of the well known picture of the so-called reinfection type was present in 18 cases. In all of these, remnants of an old first infection, namely an ossified or stony complex, were demonstrated. In 8 cases the lymph node groups regional to the progressive postprimary pulmonary lesions showed marked tubercular lesions similar to those seen in lymph nodes adjoining an active Ghon focus of first infection. The anatomical picture in the majority of the cases of this group pointed to exogenous reinfection or superinfection of the lungs. In 2 instances a healed stony complex of primary intestinal tuberculosis was found with an overwhelming recent tuberculous bronchopneumonia with extensive intrabronchial spread. This was obviously an effect of true exogenous reinfection. In 1 case only the anatomical picture suggested a continuous progressive lymphogenic spread in direct connection with a primary focus. In this case the lower tracheobronchial lymph nodes, those in the posterior mediastinum and on each side of the abdominal aorta, including especially the celiac nodes, showed extensive caseation and direct spread into both adrenal glands, causing Addison's disease.

Of the 33 cases with a relatively recent complex, in 15 progressive tuberculosis with hematogenous or intrabronchial spread had developed. In only 3 cases out of 28, progressive hematogenous or intrabronchial tuberculosis was caused by the recent complex of a secondary infection.

Many incidental findings in such cases where death was not caused by tuberculosis are of considerable significance for a better understanding of the pathogenesis of tuberculous lesions. This applies especially to the apical and subapical foci. Our material contains many cases in which these mostly single focal lesions had formed during the active stage of the primary focus. As there was no anatomical evidence of hematogenous metastases to other organs these single foci had developed either following superinfection from without or extension from the primary focus by intrabronchial spread. The

histological structure of these additional foci was identical with that of the primary focus. Cases in which primary tuberculous infection remained restricted to the parenchyma of the lungs without even microscopic evidence of spread to the lymph nodes formed about 10 per cent of our material. The incidence of true exogenous reinfections of the lung with the formation of a typical tuberculous complex of Ranke in the presence of an old, usually completely healed complex was also around 10 per cent of our material thus far examined.

### *Discussion*

(Dr. Esmond R. Long, Philadelphia, Pa.) I should like to ask if Dr. Terplan has the impression that the later primaries, even though they do involve the lymph nodes, involve them to a considerably less extent than primary tuberculosis of childhood. It is quite obvious that as we are entering a period with less and less primary infection in the earlier years, primary infection is being postponed. We have not yet studied the question enough to know exactly how it compares when first acquired in adult life with the primaries which were formerly so frequent in childhood. Postmortem material is of course selected material, in that, at least, the patient dies. There is a definite clinical impression in some quarters that late primaries behave differently from the early primaries. The early primaries, as a rule, show distinct changes in the lymph nodes. The primary infections we now see in nurses and in medical students seem to lack changes in the lymph nodes which can be seen in the x-ray film. I have no doubt these changes could be seen in the hilum nodes if we could dissect them out. It would seem as though the primaries of later life are different from those of childhood.

(Dr. Béla Halpert, New Orleans, La.) What criteria were used in differentiating morphologically between the early and the more recent lesions — those which are fairly close to one another?

(Dr. William H. Feldman, Rochester, Minn.) I should like to ask if the definite tuberculous character of the primary lesions was established by guinea pig inoculation.

(Dr. Terplan, closing.) In reply to Dr. Long's question, I should like to say this: Kuess, and later Blumenberg, are of the opinion that the tuberculous changes in lymph nodes regional to a focus of late primary infection are much less marked than those seen in primary complexes in children. The material we have examined so far during the past 7 years does not support that view. As a matter of fact, the changes in the lymph nodes in many cases of decidedly late primary infection in individuals from 20 to 40 years of age, and even in one senile patient over 80, which were found incidentally at post-mortem, were just as marked as those in children who did not die of tuberculosis. I believe that age alone is not the guiding principle in determining the extent and degree of lesions of the lymph nodes regional to the primary tuberculous focus.

In reply to Dr. Halpert, the criteria we apply for differentiating between older and more recent foci are of course only relative. A focus completely encapsulated in the calcified or partially ossified state is much older than a caseated lesion. On the other hand, we could not find any principal difference in the structure of foci of first infection and focal lesions of so-called reinfection. We were not able to support the claim of Puhl and Aschoff that single foci of reinfection always show a structure different from that of pri-

mary foci. We have shown, especially in complexes of different ages, that the focus of the second infection has exactly the same histological structure as that of the first, and that single, focal subapical lesions can look exactly like a typical primary focus with a narrow bony ring, exhibiting in sections stained for elastic fibers a caseated pneumonic structure. We are well aware that not all calcified structures, such as can be found in children, and more so in adults, are of tuberculous nature. We call a calcified focus tuberculous only if we can detect the original caseated pneumonic alveolar pattern.

In reply to Dr. Feldman, we examined many old tuberculous foci for acid-fast bacilli. We have not used calcified or ossified foci for animal inoculation. The more recent literature on this subject reveals the fact that in well controlled experiments of this kind no evidence of living tubercle bacilli in such obsolete foci was obtained. I cite only the experimental work of Schrader. All his injections gave negative results. Not even in primary foci in the chalky state did we succeed in demonstrating tubercle bacilli. We examined a number of these cases as we were interested to determine whether or not true reinfection can occur in the presence of a chalky focus. If this should be a possibility, morphologists would be inclined to speak of superinfection rather than of true reinfection. We have examined chalky foci found in children without demonstrating tubercle bacilli. In some of these cases, however, in addition a recent caseated complex of a second exogenous infection of the same anatomical type as that seen in the primary complex was found in the other lung.

THE INCIDENCE AND SIGNIFICANCE OF HEALED MILIARY TUBERCLES IN THE  
PARENCHYMATOUS ORGANS. Herbert S. Reichle and (by invitation)  
John L. Work, Cleveland, Ohio.

*Abstract.* Small spherical bodies, usually called phleboliths, were found in the liver, spleen and kidneys in 20 per cent of 500 consecutive autopsies at the Cleveland City Hospital. No condition other than parenchymatous degeneration, hyperemia and edema, primary tuberculosis and arteriosclerosis occurred in more than 20 per cent of the autopsies. In 94.5 per cent of the cases with nodules, primary or reinfection tuberculosis, healed or active, was found; in only 5 cases was evidence of a tuberculous infection lacking, and in only 1 of these had the lungs been examined by x-ray. The nodules were histologically indistinguishable from small primary tubercles or healed primary satellite tubercles and tubercle bacilli were demonstrated by animal inoculation in 3 out of 14 cases. They were found in the parenchyma of the spleen, under the capsule of the liver and in the cortex of the kidneys, in locations where large veins are not found. True phleboliths, on the other hand, were demonstrated in the hilum of the spleen, also in the periprostic veins, and their structure and relation to surrounding tissues were entirely different. These lesions are therefore to be regarded as miliary tubercles, the result of a hematogenous dissemination during the primary phase of infection. This statement is further strengthened by the fact that the incidence of these tubercles is the same throughout all age groups of our population.

In order to study a possible relation of this hematogenous dissemination to the resistance of the individual, the cases were further divided into three groups. Group I consists of cases with obsolete tuberculous lesions showing no activity microscopically; the bulk of these cases showed only one or more

components of a primary complex and this group is designated as "resistant." Group II includes the cases in which there was no clinically significant disease but in which microscopic study showed some activity; this group is designated as "moderately resistant." Group III comprises cases presenting clinically important active disease and the majority of these patients died from tuberculosis; this group is designated as "susceptible." The incidence of healed miliary tubercles in these groups was 39.2, 23.5 and 17.2, respectively, and this relationship obtains for all significant statistics in subgroups arranged according to age and color.

It is therefore concluded that the lesions described represent the result of an early hematogenous spread with limited seeding of tubercles. This acts as an autovaccination, conferring upon such individuals a resistance against reinfection which others, not showing the results of such a spread, do not have.

### Discussion

(Dr. Alfred Plaut, New York City.) I should like to know how often in this autopsy material the parasitic nodules in the liver which we are accustomed to call *Pentastoma denticulatum*, or *Linguatula rhinaria* were found. In New York I find these small nodules rather frequently, and they bear a striking resemblance to some of the pictures shown to us here. This especially refers to the liver. I have had no experience with similar nodules in the kidney, and I have seldom seen them in the spleen.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) The lesions in the spleen and liver which Dr. Reichle has shown have been found quite frequently in our postmortem material. It is my impression that they are seen more frequently here than we had seen them abroad at the Institute of Dr. Ghon. We feel as Dr. Reichle does that the majority of such calcified nodules in the spleen are tubercles and not phleboliths. We have seen these tubercles, especially in cases with hematogenous tuberculosis, in other organs. I would prefer to speak of them as hematogenous tubercles rather than as miliary tubercles. With regard to the significance of these tubercles in relation to an increase in the resistance against reinfection, we had some experience different from that of Dr. Reichle. We have seen healed calcified tubercles of the same structure as those demonstrated by Dr. Reichle in the spleen, together with remnants of an old primary complex. In some of these cases, however, there was evidence of a true exogenous reinfection of more recent nature. We find it difficult to interpret our morphological lesions in terms of different degrees or changes of "resistance." This applies especially to children. They might acquire a tuberculous lesion, a single focus, restricted completely to the parenchyma of the lung, with no spread to the regional lymph nodes. This lesion heals completely. It might remain the only tuberculous lesion throughout life. In other cases, however, in children with a similar single healing or healed focus, recent caseated lobular pneumonic foci were found in addition with masses of tubercle bacilli, spreading by the intrabronchial route.

(Dr. Morton McCutcheon, Philadelphia, Pa.) Dr. Reichle's first slide showed a good many of these little lesions in the tissue. I wonder whether he would think that solitary lesions, 2 to 3 mm. in size, in the spleen or in the liver also belong in this classification of obsolete tubercles.

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) I should like to ask whether a tuberculin test was done on these patients, especially those in Group I, and if so whether it was positive or negative.

(Dr. Otto Saphir, Chicago, Ill.) Has Dr. Reichle seen a case which showed both lesions — calcified lesions in the liver and spleen, and recent miliary tubercles?

(Dr. Paul R. Cannon, Chicago, Ill.) I should like to ask Dr. Reichle if he has seen or heard of not only calcified but ossified lesions in the spleen. Huggins has been unable to produce experimental ossification in the spleen. I wonder if you know of any instance of ossification of such nodules in the spleen.

(Dr. Virgil H. Moon, Philadelphia, Pa.) We are all familiar with the occasional occurrence of a progressive destructive organ tuberculosis, a sequel of the primary complex, which causes destruction of the adrenals, kidneys, and sometimes the liver, spleen and brain. I wonder whether Dr. Reichle would interpret the lesions he has described, and which we have all seen, as possibly healed extrapulmonary lesions of the primary tuberculous complex which, had they not healed, would have progressed to destructive caseous lesions in one organ or another.

(Dr. Reichle, closing.) Any attempt to go into all these questions in detail would be impractical. Let me assure you that I realize the difficulties, whenever a discussion concerning resistance in tuberculosis is opened. The paper I presented is more or less of a thought and not a conclusion. This problem has to be attacked from so many different angles that any attempt to solve it from one angle is quite hopeless and leads to a false perspective.

The next point I should like to call attention to is that when I talk about resistance I am obviously talking about it as a statistical problem, and on this basis there will be individual cases which always fall out of the scheme. There is no doubt about that. Whenever we talk about resistance in tuberculosis we talk about a very limited and very variable factor, and we shall find that there is evidence coming from all sides pointing to the fact that resistance to tuberculosis depends on this paradox: the spread of elements of the primary infection more or less throughout the body, without the development of tuberculous septicemia, *i.e.* miliary tuberculosis. It is my personal opinion that a primary process remaining localized will never give the resistance that a spreading one will. That was shown in Calmette's work and by a number of others. Furthermore, experimental work has shown that spread occurs very early. This work I believe was done in Saranac, and showed that if tubercle bacilli are injected into the pad of a guinea pig's foot, and amputation of the extremity is performed within a few hours, the organisms would nevertheless spread to the rest of the body even though no primary focus had yet been established. Therefore, although I agree that there are cases which will not possibly fit into this scheme, I do think that in general the spread is part of the defensive mechanism. I call your attention to the old rule of pathologists that we do not find many cases of miliary tuberculosis in individuals with extensive organ disease. Individuals with chronic pulmonary tuberculosis coming from sanatoriums do not usually show miliary tuberculosis, and on the other hand, patients with miliary tuberculosis usually have a rather small focus of disease somewhere in the body.

Concerning Dr. Plaut's question, we have seen parasites in the liver but do not believe these lesions are the result of a parasitic involvement. Possibly a parasitic infestation might heal in such a fashion.

We have seen cases where there were only one or two, or perhaps a single tubercle, in the spleen. Let me call your attention to the fact that all calcified



lesions are not tubercles. In every single case some of the lesions were examined microscopically, and if we found the architecture described, then we included the case in our group.

To Dr. Clawson I regret to say that we have no knowledge concerning the tuberculin test. These were routine autopsies.

Dr. Saphir asked about the combination of miliary tuberculosis and these lesions. We have 2 cases in children with progressive primary tuberculosis who showed fresh tubercles and these older miliary lesions. Dr. Terplan is justified in objecting to the term miliary. Hematogenous would be better.

To the best of my knowledge we did not see ossified tubercles in the spleen; they were, however, found in the liver.

In reply to Dr. Moon, permit me to emphasize that we regard these lesions as instances of hematogenous tuberculosis.

THE OCCURRENCE OF VIRULENT TUBERCLE BACILLI IN PRESUMABLY NON-TUBERCULOUS TISSUES OF THE LUNG. William H. Feldman and (by invitation) A. H. Baggenstoss, Rochester, Minn.

*Abstract.* Opie and Aronson having reported the demonstration of virulent tubercle bacilli in the presumably non-tuberculous lung tissue in 15 of 33 bodies examined in Philadelphia in 1927, a comparable study was made of material secured at Rochester, Minnesota. Tissues from 51 unembalmed bodies were utilized for the inoculation of guinea pigs. The age distribution was from 2½ to 93 years, with the largest number of cases in the fifth and sixth decades. All were white. Thirty-four were males and 17 females. The bodies selected for the study represented individuals who with one exception had died of causes other than tuberculosis. In 12 of the bodies no gross or microscopic evidence of tuberculosis was found, while in 38 there were lesions of latent or healed tuberculosis. In the majority of instances the signs of primary tuberculosis present were those of the primary complex of the lungs.

Material for the inoculation of guinea pigs consisted of what appeared to be non-tuberculous portions of the upper and of the lower lobes of each lung and the apparently non-tuberculous hilar lymph nodes. In all but 3 cases three emulsions of tissue were prepared from each body and used to inject 6 guinea pigs. A total of 150 emulsions was utilized to inject a total of 300 animals.

Positive results were obtained from only 3 cases and since in 1 of these the cause of death was tuberculous enteritis and peritonitis, only 2 positive cases need be considered. In 1 case tubercle bacilli definitely identified as bovine in type were obtained from the spleen of 1 of 2 guinea pigs previously inoculated with a composite emulsion prepared from presumably normal tissues from the parenchyma of the upper and lower lobes of the left lung. In another case tubercle bacilli were demonstrated from the hilar lymph nodes.

The results of this study made of material from an area where the tuberculosis morbidity is not high indicate that virulent tubercle bacilli are infrequently present in the presumably non-tuberculous tissue of the lungs of individuals dying of causes other than tuberculosis.

*Discussion*

(Dr. Hans P. Popper, Chicago, Ill.) I should like to ask whether in these cases the blood and other organs were examined for tubercle bacilli. While

in Vienna we examined the blood, spleen, liver and kidneys in cases of fatal and non-fatal tuberculosis, and were able to prove fairly often the presence of tubercle bacilli by guinea pig inoculation and culture.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I should like to add that the positive result in 1 case of Dr. Feldman's, in which tuberculous enteritis was present, is in line with a number of experiments we carried out after injecting material from lymph nodes from cases of Hodgkin's disease. Although such nodes had not exhibited tuberculous lesions, we produced tuberculosis in guinea pigs whenever they were from cases in which marked active tuberculosis was combined with Hodgkin's disease, especially in the fulminating miliary type seen in adults. With regard to the other positive result in Dr. Feldman's material, I am inclined to believe that its source might have been a small active tuberculous lesion in a bronchomediastinal lymph node. These tuberculous lesions in bronchomediastinal lymph nodes are quite frequently found in older individuals. It was thought that they developed and spread following lymphoglandular exacerbation. Sometimes they are small enough to be overlooked with the unaided eye.

(Dr. Herbert S. Reichle, Cleveland, Ohio.) I would like to emphasize what Dr. Terplan says. I think possibly the difference between the results obtained by Feldman and by Opie are due to the fact that there may have been quite a number of patients with endogenous reactivation in Opie's group. I agree with Dr. Terplan. In the City Hospital we see an unusual number of cases of tuberculosis in individuals who come from the Psychopathic Division or the Tumor Division. I think that the endogenous source of tubercle bacilli cannot be excluded.

(Dr. Feldman, closing.) In reply to Dr. Popper, we did not examine the blood or other organs. We limited the investigation entirely to the lungs and the contiguous lymph nodes.

STUDIES OF THE CHEMOTACTIC PROPERTIES OF TUBERCULOPHOSPHATIDE AND TUBERCULOPOLYSACCHARIDE. William B. Wartman and (by invitation) E. S. Ingraham, Jr., Cleveland, Ohio.

*Abstract.* In experiments reported before this society 2 years ago it was shown that *in vitro* tuberculoprotein strongly attracted human polymorphonuclear leukocytes. In the present experiments the chemotactic properties of the phosphatide and polysaccharide fractions of the tubercle bacillus were studied. In concentrated form the tuberculophosphatide was found to be toxic for both human and rabbit neutrophils, but in suitable dilution it caused weak attraction of these cells. The tuberculopolysaccharide caused weak negative chemotropism of the leukocytes.

#### Discussion

(Dr. Morton McCutcheon, Philadelphia, Pa.) I wonder whether Dr. Ingraham has any information about the adsorption of these carbohydrates on kaolin. That is of quite considerable interest to us.

(Dr. Esmond R. Long, Philadelphia, Pa.) Did Dr. Wartman and Dr. Ingraham carry out experiments with leukocytes in both normal and tuberculous animals, and if so, I would be interested to hear if there was a difference in the results.

(Dr. Ingraham, closing.) We did not mean to claim the polysaccharide was

adsorbed. We only used the inert substance as opaque material, and the particles were not washed. They were merely saturated with dilute solution.

In reply to Dr. Long, we did not use tuberculous animals.

A STUDY OF PURIFIED ANTIGENS IN RELATION TO VIRULENCE OF ROUGH AND SMOOTH STRAINS OF *SALMONELLA AERTRYCKE*. G. M. Mackenzie and (by invitation) R. M. Pike and R. E. Swinney, Cooperstown, N. Y.

**Abstract.** From 2 rough and 2 smooth strains of *Salmonella aertrycke* polysaccharides were prepared by the method of Raistrick and Topley. One of the smooth strains is highly virulent, the other is of very low virulence. The rough strains are avirulent. The polysaccharides from the 2 smooth strains have the same toxicity and immunizing power for mice; analysis of their antigenicity shows them to be complete antigens and serologically indistinguishable. The polysaccharides from the rough strains are much less toxic and possess little or no immunizing power; they too are complete antigens. The polysaccharides from the smooth strains and those from the rough strains show complete antigenic disparity. The results indicate: (1) the smooth somatic polysaccharide is not the major determinant of virulence in *Salmonella aertrycke* infection in mice; (2) in confirmation of the work of Raistrick and Topley, and that of Boivin and collaborators, however, the smooth somatic polysaccharide has been found to be chiefly responsible for immunizing power; (3) the virulence of this species of microorganism is probably determined by serologically inactive components of the bacterial cell; and (4) a polysaccharide, which is also a complete antigen, is present in rough avirulent strains of *Salmonella aertrycke*.

*Discussion*

(Dr. A. B. Wadsworth, Albany, N. Y.) Was there much difference in the nitrogen content — total and amino — in the rough and smooth preparations?

(Dr. Mackenzie, closing.) Quantitative analyses have been made of these preparations; the nitrogen content is 4.5 to 5 per cent. On hydrolysis the yield of glucose is about 40 per cent from the smooth strains, and 20 per cent from the rough strains. There are some differences in the quantitative elementary analysis, but the chief difference between the smooth and the rough strains is in the yield of glucose on hydrolysis.

A STUDY OF TWO STRAINS OF *B. VIOLACEUS* ISOLATED FROM HUMAN BEINGS. Malcolm H. Soule, Ann Arbor, Mich.

**Abstract.** A strain of *B. violaceus* was isolated in pure culture at autopsy from the blood, spleen and liver of a girl 15 years of age. Nine months before death a large fluctuating mass in the right side of the neck had been treated surgically and *B. violaceus* was the only viable organism in the aspirated pus. The incision did not heal and roentgen therapy over a period of months merely held the lesion in check; it continued to drain although the patient was otherwise in excellent physical condition. An exploratory biopsy was diagnosed as mixed tuberculous and pyogenic infection. Clinically the case was one of tuberculous adenitis. Cultures and guinea pig inoculations were negative for the tubercle bacillus. A series of tuberculin tests was nega-

tive. Fluoroscopic examinations and x-rays of the chest were negative. Repeated blood cultures were negative; the blood serology was negative for syphilis, *B. abortus*, and *M. melitensis*, *B. tularensis*, *B. typhosus*, and *B. paratyphosus A* and *B*. The report on the gross pathology showed active chronic tuberculosis of the base of the left lung, tuberculous abscesses of the left leaf of the diaphragm; a large chronic tuberculous abscess of the left hypochondrium; an extensive hematogenous chronic tuberculous abscess of the liver; and necrotizing miliary tuberculosis of the spleen, liver and lungs. The histological studies revealed no tubercles, only a septicopyemia.

A comparative study of the morphology, cultural characteristics, biochemical reactions, serology and pathogenicity of this human strain, labelled Human Strain 1, with two old laboratory strains of *B. violaceus* was completed when Black and Shahan reported the isolation in pure culture of a similar organism from purple pustular areas surrounding anthrax-like lesions on the arms and chest of a boy of 6 years. After 2 months these lesions apparently healed and the patient was lost sight of until 13 months later when he appeared with a severe cervical adenitis, high fever and marked prostration. The adenitis subsided and the body became covered with lesions ranging from minute vesicles to large gangrenous areas. Death occurred 15 months after the original observation. Unfortunately no autopsy was granted. The only organism ever found was *B. violaceus*. The same serological and cultural studies mentioned in connection with the young girl were carried out by Black and Shahan with identical results. In addition, they found the blood serum of the body agglutinated the homologous strain of *B. violaceus* in a dilution of 1:1280.

The Shahan strain was added to the comparative studies under the designation Human Strain 2. Morphologically the four strains were identical. There were cultural differences particularly as regards the resistance to aniline compounds. H 1 was sensitive to the concentrations of dyes ordinarily employed in mediums for the isolation of acid-fast organisms; the others were not. A deep violet color was associated with the growth of each strain. All bacilli in a given culture did not produce the same amount of pigment. Strain-specific antibodies were elicited by the injection of rabbits with suspensions of the dead cells. H 2 induced a progressive infection on intraperitoneal injection in rabbits and guinea pigs. Broth suspensions of the four strains were toxic for animals in quantities of 0.5 cc.

In consideration of the aforementioned observations, the wide distribution of *B. violaceus* in air, soil and water should be envisioned as potentially dangerous. If found in diseased processes, this organism should be carefully investigated rather than treated as a contamination.

### Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I was responsible for the incorrect diagnosis of mixed tuberculous and pyogenic infection which was made on the biopsied lymph node of this patient, and being fully aware of the subsequent bacteriological investigation, I have reviewed these slides from the standpoint of pathology and cannot see how I could have made any other diagnosis. We have, however, stained not only the same blocks of tissue, but many others from the autopsy in this case, and we have failed to demonstrate acid-fast bacilli by staining tissue sections. As the matter now stands, it is a new entity to me in my experience as a pathologist.

(Dr. Clayton E. Royce, Jacksonville, Fla.) The *B. violaceus* in the 1st case described was found in my laboratory at St. Vincent's Hospital in Jacksonville, and as the author said, we did not feel it would be justifiable to connect it with the disease present in the young woman, although it was found several different times in culture, and no other organisms were demonstrated. I am very glad to be here and to hear the careful analysis which subsequently was made of the organisms recovered from the patient.

(Dr. Howard T. Karsner, Cleveland, Ohio.) I noticed in the autopsy report in the 1st case, that of the young girl, that there was a gross anatomical diagnosis of tuberculosis, and that it was not confirmed by microscopic examination. I think it would be of interest if we knew something about the microscopic appearance of the nodules which in gross appeared to be tuberculous.

(Dr. Paul Klemperer, New York City.) Was the organism found in the tissues? The question which Dr. Karsner asked would be interesting to hear about.

(Dr. Soule.) I can comment on Dr. Royce's remarks, namely that the bacteriological work done in his laboratory was most thorough and of the highest quality. In our own studies *B. violaceus* was never found until autopsy because the pus and biopsy specimens were treated with 4 per cent potassium hydroxide previous to the inoculation of laboratory mediums. This is the routine technique in the isolation of the tubercle bacillus. We also found this strain sensitive to the dyes employed in acid-fast mediums. This was not true of the other strains investigated. The other questions I cannot answer.

(Dr. Weller.) I am sorry I have not refreshed my mind recently enough to give the detailed answer to these questions that they deserve. In the first place I think the prosector at the time of the autopsy was somewhat influenced by the biopsy diagnosis, so that he was quite satisfied with the diagnosis of generalized tuberculosis.

(Dr. Frank B. Lynch, Philadelphia, Pa.) Was there any study of this tissue made from the standpoint of *Coccidioides immitis*? Dr. Soule answered the question that it was not cultured in his laboratory. I wonder if that has been thought of in a study of the tissues.

(Dr. Weller.) With the very complete study we made of this case, if there had been *Coccidioides immitis* we would have been aware of that fact. There was no indication whatever of *Coccidioides immitis* infection. We are quite aware of the necessity of looking into that group in all the tuberculous-like material that comes to our department.

(Dr. Karsner.) Mr. President, could your remarks in regard to the microscopic appearance be expanded a little when the material is ready for publication in the Proceedings?

(Dr. Weller.) I will be glad to cooperate with Dr. Soule in that respect.

*(Prepared Statement by Dr. Weller)*

"The first biopsy specimen showed an area of combined suppuration and caseous necrosis. There was no tubercle formation but the border of the necrotic area was composed in part of epithelioid cells with many large mononuclear phagocytes. This zone was relatively avascular. It was thought that even in the absence of definite tubercles, a diagnosis of mixed tuberculous and pyogenic infection was justified. A second biopsy, taken about 1 month later, showed substantially the same changes, but with even more caseous

necrosis. At the autopsy, focal necrotizing lesions were found in the lungs, spleen, liver and pelvic peritoneum. The prosector made a diagnosis from the gross appearances only of active chronic tuberculosis of the base of the left lung, chronic tuberculous abscess of the left hypochondrium and generalized miliary tuberculosis. Microscopic examination showed these lesions to be essentially pyemic abscesses, but even microscopically the purulent exudate and tissue debris in the interior of each of them showed changes closely approaching caseous necrosis. The borders of the abscesses were made up of granulation tissue which was but slightly vascular and rich in large mononuclear phagocytes. No tubercles were found and no multinucleate giant cells. The various higher fungi were searched for and none was found. Staining for tubercle bacilli gave negative results on the biopsy material and also on selected blocks from the material obtained at autopsy. Likewise cultures for tubercle bacilli were negative, as was also animal inoculation. The recovery of *B. violaceus* from blood from the heart, from exudate from the lesions in the neck, and from pyemic abscesses in the liver has been described by Dr. Soule."

METHODS WHICH MAY AID THROUGH CORRELATION IN EXPLAINING PHYSIOLOGICAL AND PATHOLOGICAL SEQUENCES OF EVENTS IN THE SPREAD OF INFLAMMATION. John W. Williams, Cambridge, Mass.

*Abstract.* New methods (*Am. J. M. Technol.*, May, 1939, 5, 68-71) show a decrease in Eh for 45-90 mm. in depth in gel mediums autoclaved and solidified in 16 by 200 mm. test tubes and set aside for periods up to 2 weeks (0.08 to -0.15v. from top to 45 mm. 1 minute after insertion of electrodes, using a Beckman potentiometer with a calomel half cell reference electrode and a medium containing 2 gm. nutrient broth Difco, 7.5 gm. agar, 1 gm. dextrose, 0.48 gm. sodium hydroxide, 1000 cc. water). Controls of agar and sodium hydroxide show these can account for the greater part of the Eh change. More acid mediums shift the curve generally into a more positive Eh range. The pH does not show significant variation with depth (8.3-8.4) in above. At depths greater than 45 mm. the curve shows some fluctuation, but not a decrease trend. Mediums placed under 60 pounds of nitrogen 4 days show a slightly greater Eh (0.11 to -0.13v. from top to 45 mm.) and slightly greater decrease in pH (7.8-8.36). Mediums placed under 60 pounds of oxygen 4 days show a greater shift of the curve to the right or a more positive Eh (0.226 to -0.142 from top to 75 mm.) and a decrease in pH (7.2-8.74). Mediums placed under 60 pounds of carbon dioxide show shift to the right but lower Eh than oxygen (0.15 to -0.128v. from top to 90 mm.) with greater decrease in pH (6.0-8.34): there seems a more gradual decrease in Eh with possibly greater penetration of carbon dioxide possibly related to decrease in acidity.

Change with nitrogen and carbon dioxide in large part may be dependent on the increase in acidity. Greater increase in Eh under oxygen indicates an effect of oxygen independent of pH. The importance of penetration of gas and resistance to this is indicated by the fact that the low Eh noted in mediums in the atmosphere is eventually reached in tubes placed under carbon dioxide and oxygen. In previous papers (*Am. J. M. Technol.*, 1938, 4, 58-61; *Am. J. Path.*, 1938, 14, 642-645; and *Growth*, 1939, 3, 21-33) variations in the depth of growths of microorganisms in shake cultures corre-

sponding to Eh and oxygen tension variations described here were reported. Gelatin and horse blood clot also show some variation of Eh; consistent and experimentally sufficiently marked effects have not yet been demonstrated for an inorganic gel such as silica gel.

Since our bodies are fundamentally organic gel in consistence this work suggests the gel may function as a tramway for a gradient of Eh and possibly pH. It may serve as a structure on which variations in physiology and pathology occur. While the gas tensions used are large, smaller variations between the blood stream and metabolizing tissues, and between normal and pathological tissues may be assumed to act likewise. Just as bacteria (anaerobes and aerobes) when planted in shake cultures grow in positions where physicochemical conditions for growth are optimum, so the cells of our body may select optimum and appropriate sites for growth. Various diseases modify conditions which the body attempts to remedy. Since practically all appearances produced by the colloidal chemist by inorganic precipitates in gels have been produced by growth of microorganisms, a suitable site dependent on physicochemical conditions and essential nutrient for proliferation and existence of our cells can be logically postulated.

A STUDY OF EXPERIMENTAL NEPHRITIS IN THE HORSE. Joseph Schleifstein  
(by invitation), Albany, N. Y.

*Abstract.* A study has been made of the renal lesions of 168 horses under experimental conditions of immunization with toxic injections of bacterial cultures and toxins in the production of therapeutic serums.

The changes in the kidney under certain conditions correspond to the following lesions observed in man: (1) acute glomerular congestion, (2) acute diffuse glomerulonephritis, and (3) focal or embolic glomerulonephritis.

(1) Acute glomerular congestion was observed in horses that died from acute shock a few hours after an injection of bacterial culture or toxin. The glomerular tufts were so distended that they completely filled the capsule and the capillaries were packed with red cells.

(2) The changes in the kidney were those of the acute diffuse glomerulonephritis that occurs in man when the horses lived for from 3 days to 5 weeks after the last injection of bacterial culture or toxin. These acute changes did not progress to chronicity. The horses were relatively highly immunized.

(3) The focal or embolic glomerulonephritic lesions of man as described by Löhlein and Baehr occurred in 16 of 20 animals with endocarditis and bacteremia. All stages of the lesion, from early glomerular necrosis to healing and fibrosis, were observed.

*Discussion*

(Dr. William H. Feldman, Rochester, Minn.) I am curious to know if the material was examined for amyloid.

(Dr. Irving Graef, New York City.) I should like to ask if Dr. Schleifstein has encountered any of the so-called embolic lesions in animals that did not have endocarditis.

(Dr. Herbert Fox, Philadelphia, Pa.) This paper seems to have two very distinct significances. The fact that glomerular lesions are found under these circumstances is rather unusual for spontaneous nephritis in the group of

animals to which the horse belongs. In the spontaneous nephritis of that group more often it is the parenchyma which suffers and the glomeruli do not, and that seems to strengthen the fact that there is in this experimental procedure some value in the production of nephritis. The secondary stages are much more interstitial, surrounding the tubules rather than around the glomeruli.

(Dr. Paul Klemperer, New York City.) I wonder what was the distribution of the lesions in respect to the organisms used for immunization.

(Dr. Schleifstein, closing.) In regard to Dr. Feldman's question, these kidneys were all carefully stained for amyloid and amyloid was not present.

In reply to Dr. Graef, there was one instance where an embolic lesion was present where there was no endocarditis, but in this there was a thrombosis of the iliac arteries.

I appreciate very much the comment of Dr. Fox on spontaneous lesions.

In reply to Dr. Klemperer, I may state that generally speaking the streptococcus showed the most severe glomerular lesions, then the meningococcus and the pneumococcus. In the toxins the lesions were not so severe as with the streptococcus.

EXPERIMENTAL PERITONITIS: ALTERATION OF THE LEUKOCYTIC RESPONSE AFTER REPEATED INJECTIONS OF PHYSIOLOGIC SALINE. Dale Rex Coman (by invitation), Philadelphia, Pa. (Presented by Dr. Morton McCutcheon.)

*Abstract.* Experimental peritonitis was produced by the method of de Haan, which consists in injecting physiologic saline into the peritoneal cavity of rabbits; after several hours the fluid is withdrawn and it then contains large numbers of leukocytes, nearly all of them polymorphonuclears. It is not understood why saline solution causes exudation of leukocytes, since sodium chloride is usually not chemotactic for these cells. Experiments were made to find whether exudation is due to impurities in the saline solution, and, more generally, whether emigration of leukocytes is always the result of chemotaxis or, on the contrary, whether it may occur in the absence of chemotactic substances.

One series of rabbits was injected with saline made with distilled water prepared in the ordinary way, in a copper still, and stored in a large tank. Another series was injected with saline made with doubly distilled water, freshly prepared, with every precaution to ensure sterility and purity. None of these rabbits had been injected previously. Cell counts showed small to moderate numbers of leukocytes in the peritoneal fluid of both series of rabbits, and there was no significant difference between counts in the two series.

Several days later the rabbits that had been injected with ordinary saline solution received a second injection, half of them again with ordinary saline and half of them with saline prepared with redistilled water. There was now a striking difference between the two series: rabbits injected with ordinary saline showed a four-fold increase in leukocytes, while those injected with specially pure saline had about the same number of cells as on the first injection. The reactivity of the peritoneal vessels had apparently become increased, so that differences in the effects of the two solutions, masked on the first injection, became obvious on the second. This difference in the effects of the two saline solutions appeared to be due to the presence of chemotactic



substances in the ordinary saline, and absence of such substances in specially purified saline, as demonstrated by experiments *in vitro*. It is concluded that exudation of polymorphonuclear leukocytes may take place in the absence of substances having demonstrable chemotactic effect, but when chemotactic substances are present, emigration is greatly increased.

### Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) This field of study is extremely interesting. Maltaner (*J. Hyg.*, 1921, 19, 309) repeated the experiments of Buchner on positive and negative chemotaxis. He found that irrespective of the agents that were used in the early experiments of Buchner the positive or negative so-called chemotactic reactions took place, simply according to the concentration of the salt solution. I think the experiments were done in capillary tubes under the skin and in the peritoneum, and the leukocytes penetrated the capillary tubes that contained the experimental fluids, or did not penetrate, according to the concentration of salt solution, — whether the density of the solution was greater or less than that of the body fluids.

(Dr. Sheldon A. Jacobson, Brooklyn, N. Y.) I should like to ask what the interval of time was between the two injections. If it was not excessively great, I wonder whether the author would not consider, in addition to his own interpretation, the following incidental factor in the obtaining of these results. Whatever this chemotactic principle was, whether it was an additional substance or saline, it evidently acted as an irritant. The method of fixation by irritation has been very well demonstrated in a series of papers by Menkin, and I wonder whether it is not possible that the first injection fixed the irritation in the lymphatics and local tissues around the peritoneum, so that when the second injection occurred, the irritant was unable to escape and was confined *in situ*, and therefore had a more pronounced effect.

(Dr. McCutcheon, closing.) I thank Dr. Wadsworth for his comments.

In reply to Dr. Jacobson, the time in these experiments between injections varied from 2 days to about a week. However, in Hamberger's earlier experiments he usually allowed 2 weeks to elapse between injections. I think Dr. Jacobson's suggestion is very interesting.

### EXPERIMENTAL ARTHRITIS IN MICE PRODUCED BY FILTRABLE PLEUROPNEUMONIA-LIKE MICROORGANISMS. Albert B. Sabin (by invitation), New York City.

*Abstract.* Two strains of filtrable, pleuropneumonia-like microorganisms, recently isolated from mice, were found to possess specific tissue affinities of such a nature that they can give rise to two experimental diseases in mice, which in some respects resemble rheumatic fever and rheumatoid arthritis in man. Strain A can multiply in the brain and in association with the cells of the parietal and visceral peritoneum, pleura and pericardium, with the elaboration of a specific exotoxin, having a special affinity for the cerebellum, which either kills the mice within a few hours or leaves them suffering from a choreiform syndrome. This toxin is also produced *in vitro* in cultures, is heat-labile, and can be neutralized by a specific antitoxin. When the rapid toxic death which usually follows the intravenous injection of the culture is prevented by the use of older mice or by inoculation of centrifuged microorganisms, about 30 to 40 per cent develop a migratory polyarthritis, during

the course of which some of the mice exhibit choreiform signs. Most of the mice tend to recover from the arthritis produced by Strain A.

Strain B, on the other hand, has a specific affinity for the joints (it does not multiply in the skin or subcutaneous tissue, brain, viscera, or their linings), in which it gives rise to a chronic progressive proliferative arthritis which clinically and pathologically bears a marked resemblance to rheumatoid arthritis in man. Arthritis was produced in practically 100 per cent of mice when 0.5 cc. of a 24 hour culture was injected intravenously, or 1 cc. intraperitoneally. It has also been possible to obtain arthritis in a small number of mice which developed a focal infection after inoculation into the vitreous of the eye. After a preliminary phase, during which the arthritis is migratory, the process becomes progressive and chronic in one or more joints, leading to ankylosis (especially in the knees) in about 70 per cent of mice. The affected animals appear otherwise healthy and not one of 150 with joint involvement (B strain) has as yet died of the infection. The microorganism has been cultivated (by the method of "blind passage") from affected joints as late as 70 days after inoculation. Pathological changes are limited to the joints and consist predominantly of proliferation in the synovial membrane, the capsule, and the perichondrium of the articular cartilage, with similar changes in the subchondral epiphyseal marrow. Rabbits and guinea pigs were not susceptible. Filtration through gradocol membranes indicated that the smallest unit of these microorganisms is not much larger than vaccine virus. The two strains possess a common antigen but are immunologically distinct.

### *Discussion*

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) The similarity of the inflammatory reaction shown here is certainly great when compared with that of acute rheumatic fever. I should like to ask Dr. Sabin if he examined the heart valves in these cases.

(Dr. Sheldon A. Jacobson, Brooklyn, N. Y.) Have Dr. Sabin's experiments lasted long enough for him to be able to state whether the disease in these animals shows any tendency to successive periods of remission and exacerbation?

(Dr. S. A. Goldberg, Newark, N. J.) I am very much impressed with the similarity of the lesions shown by Dr. Sabin to those we studied in arthritis in animals in 1917. We found certain strains of *B. coli* and streptococci in the lesions.

(Dr. Carl V. Weller, Ann Arbor, Mich.) Chondroplastic synovial villous processes are seen in human pathology. I remember in the first year or two in which I was in pathology a surgeon brought into the laboratory about a pint of round firm bodies, and with an assumption of learning that was unjustified, I told him they were corpora oryzoidea. When we examined them microscopically, there was not a single rice body; we found only small spherical nodules of cartilage — detached chondroplastic synovial villi. These pictures brought that early experience to my mind.

(Dr. Sabin, closing.) I expected Dr. Clawson's question, and if I may have a minute to show the last two slides which I did not have time for, they might answer his question. I have not yet had time to make a complete study of the manifestations in the hearts of the mice injected with Strain A, but I have found two things which I want to show. Some of the myocardial

vessels exhibit a subendothelial infiltration of this sort (illustrating on screen) with a palisading epithelioid type of cells, which I have not yet observed in the normal, but I am not yet ready to assign any significance to it.

In regard to the question of a change in the mitral valve, I may say that in the same heart that exhibited the vascular changes the mitral valve was thickened, both leaflets showing many mitotic figures, which may be indicative of active proliferation, or may be normal. I do not know. I have not yet found it in the normal. Further studies may reveal more.

Dr. Jacobson inquired as to remissions. During the first month of the arthritis there is a migratory phase with both strains. The process begins in one or two joints, may then leave them and appear in others, and sometimes there may be a short period of about a week when all the joints have cleared up, and then they may come back again. With Strain A, 90 per cent of the mice seem to be rid of their arthritis after that time, whereas with Strain B it settles down in one, two, or more joints, and progresses to a condition where many of the mice are quite crippled with extensive ankylosis.

Dr. Goldberg mentioned arthritis in animals in which other bacteria were found. Whether or not the *B. coli* or streptococci were etiologically related to the lesions he saw, we all know that experimental arthritis in one form or another has been produced with various bacteria. Our interest in the particular group of microorganisms I just described is that they have specific tissue affinities. On the one hand we have a strain which inoculated into any other part of the body will do nothing. When it localizes in the joint it is capable of carrying on certain processes which lead to a chronic, progressive proliferative arthritis. Another strain has somewhat broader affinities but is still restricted to tissues of mesenchymal origin. It may be just another group of microorganisms which we have not known before producing something which is similar to certain manifestations of human disease, and may not have anything to do with the actual etiology of the human conditions, but as far as I know, the experimental syndromes I have described are quite different from anything that one has been able to produce with the known pyogenic microorganisms.

I can only thank Dr. Weller for his discussion.

TRANSMISSION OF ENDOCARDITIS LENTA TO RABBITS. Ward J. MacNeal and (by invitation), Martha Jane Spence and Marie Wasseen, New York City.

*Abstract.* Endocarditis has been produced by injury to the heart valves followed by infection with various bacteria in experimental animals by many investigators since the pioneer work of Rosenbach in 1878. Dreschfeld in 1887 succeeded in transmitting the disease to rabbits by simple intravenous injection of pure cultures and this was confirmed by Horder in 1907. The present authors have been able to transmit to rabbits by repeated intravenous injection of serum-broth cultures of *Streptococcus viridans* the specific type of vegetative endocarditis due to this organism and best designated at present by the name endocarditis lenta. This we regard as production of the disease by pure cultures in the bacteriological sense of Robert Koch.

*Discussion*

(Dr. Joseph Tannenberg, Albany, N. Y.) In rabbits which by repeated injections (6 to 9) had been immunized against horse serum or pneumococci or streptococci small petechial hemorrhages were frequently found within the tissue of the mitral valves. I would like to inquire if Dr. MacNeal has also seen such lesions in animals which perhaps were sacrificed in the early stages of his experiments. Such lesions might possibly form the local basis for the endocarditis obtained in the end-stage of these experiments.

(Dr. A. B. Wadsworth, Albany, N. Y.) Dr. Tannenberg has stressed the point which I think is crucial in this conclusion of Dr. MacNeal's: that the induction of endocarditis experimentally does not depend upon damage to the valves. Of course the injection of this material introduces an artifact and the possibility of local injury. That was quite commonly observed in studies on the development of endocarditis in immunized animals which I published some years ago. In those animals I pointed out that there was in the primary lesion a hemorrhagic extravasation, an action of the toxins on the blood vessels, as the primary predisposition to the lesion. Furthermore, these lesions occurred in the process of immunization. The streptococci, pneumococci and meningococci give rise to this experimental endocarditis in horses under immunization. The action of the streptococcus toxin is possibly very definitely dependent on a condition of tissue susceptibility, sensitization, or possibly the specific sensitization of immunization, a partial state which I reported in 1918 (*J. A. M. A.*, 1918, 71, 2052). It has come forward in the studies of the relation of the streptococcus to scarlet fever—a selective action on the blood vessels. With the introduction of this culture material, I wonder if you could exclude the possibility of the action of these toxic injections on the blood vessels being a primary injury, and predisposition to the localization of the microorganisms in the valves. That is what appears to take place in these very common endocardial lesions that we get in horses under immunization with streptococcus, with pneumococcus, and even with meningococcus.

(Dr. B. J. Clawson, Minneapolis, Minn.) I should like to ask Dr. MacNeal if he studied the myocardium.

(Dr. Otto Saphir, Chicago, Ill.) I should like to ask Dr. MacNeal why he calls this condition endocarditis lenta rather than infective endocarditis, or acute bacterial endocarditis.

(Dr. MacNeal, closing.) In regard to the early lesions, that of course we cannot answer, because we have not killed these animals a few days after inoculation, but have allowed them to develop the disease, and the animals have gone on to a natural death. So the lesions have not been early lesions. The animals have died after several days or weeks or months. I cannot answer that question; there are a great many problems arising which would require further study.

In regard to the question of local injury, it seems that is a point which perhaps can be studied, but I do not know of any exact observations which would bear on it. However, when one inoculates any microorganisms into the body, it is difficult to exclude the action of some injurious agent. The point I wish to make here is that by a simple technic, lacking in modern refinements, but similar to that used in the beginning days of bacteriology, we have been able to transfer a disease to the rabbit just by taking a serum-broth culture of the causative microbe and repeatedly injecting it into the ear vein.

In regard to the myocardium, I would say there are lesions in the myocardium. I am not in a position to make any more definite statement at this time. I should be delighted to submit some of these sections to Dr. Clawson, if he is interested.

The question as to why we chose to call the disease endocarditis lenta is raised. I believe this is important. I think we are now at a point where we should cease to talk about endocarditis as a single disease entity. We should get down to brass tacks in regard to the etiology, and I choose to use the term endocarditis lenta here because it more nearly represents the designation of a specific infectious disease due to a specific type of microorganism than any other term which is commonly employed. I do not mean here the causation of an endocarditis by some cocci or some bacilli, but I mean a specific infectious disease due to a particular type or group of streptococci, which is the common cause of this disease in man. I believe it is time for us to give a specific etiological diagnosis to lesions of heart valves, according to the specific microbic cause, just as, instead of talking about granuloma, we should distinguish between syphilis, tuberculosis, leprosy, and a foreign body reaction.

EXPERIMENTAL PNEUMOCOCCIC MENINGITIS. (a) PERMEABILITY OF CEREBROSPINAL BARRIER TO ANTIBODIES. (b) LACK OF IMMUNITY OF RECOVERED ANIMALS. Paul Gross and (by invitation) Frank B. Cooper, Pittsburgh, Penn.

*Abstract.* Type I horse and rabbit antipneumococcic serums were effective in experimental Type I pneumococcic meningitis in rats. This suggests that the cerebrospinal barrier is more permeable in the rat than in the dog or man. Type III rabbit antipneumococcus serum, while effective in experimental Type III pneumococcic pneumonia in rats and Type III pneumococcic sepsis in mice, was without demonstrable therapeutic action in Type III pneumococcic meningitis in rats. This ineffectiveness of the Type III rabbit antipneumococcus serum is interpreted as indicating an antibody too large to pass the cerebrospinal barrier.

Rats which recovered from pneumococcic pneumonia showed a fair degree of immunity to subsequent intraperitoneal infection with one to ten fatal doses of the homologous strain; whereas rats similarly recovered from pneumococcic meningitis showed no immunity.

### Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) In my early experiments on the action of serum on pneumococcus infection in rabbits I worked with the homologous rabbit serum and I was unable to obtain any survival by the administration of serum 4 hours after the intravenous injection of pneumococci. In these experiments the inoculation is a local one. The serum was administered 6 hours later and a variable number of the animals survived. The question arises as to localization of the infection in each instance, that is, how soon the pneumococci arise to a generalized bacteremic infection. I wonder whether or not this might have been an important determining factor in the survival or death of the animals in these experiments.

(Dr. Gross.) In answer to that question, the evidence which we have is to a large extent circumstantial. We have made a study of the residual lesions in the recovered animals. This study involved a series of about 70

animals, and in every one of these recovered animals we found residual lesions. They were of variable degree and distribution. We found generalized, but not uniformly distributed thickening of the meninges. We found lymphocytic infiltration. We found various types of cortical lesions, not only over the cerebral hemispheres, but also the cerebellum. We also found less pronounced meningeal changes in the spinal cord.

(Dr. Wadsworth.) I meant generalized bacteremia.

(Dr. Gross.) Bacteremia was present in 100 per cent of the animals tested 4 hours after the infection in a previous analogous series.

THE INFLUENCE OF SULFANILAMID UPON THE EVOLUTION OF EXPERIMENTALLY-INDUCED PNEUMOCOCCUS PNEUMONIA IN RATS. David Goldstein (by invitation) and Irving Graef, New York City.

*Abstract.* Following the technic of Nungester and Gunn, and Mellon and his associates, we have introduced a mixture of gastric mucin and pneumococci into the bronchial trees of rats, inducing a pneumonia of lobar proportions which bears a striking resemblance to the gross and microscopic picture observed in man. Culture dilutions of 1:10 of a single Type III strain were used to provide an inflammatory pattern in 80 albino rats upon which the influence of sulfanilamid was studied.

Animals in the treated groups were given sulfanilamid (Winthrop Chemical Company) in olive oil emulsion in daily doses of 0.75 mg. per gm. of rat by subcutaneous route. The first administration of the drug was made within 2 hours of the operation and repeated once daily. Except for animals in survival experiments, the rats were divided in groups and sacrificed serially at daily intervals. Just prior to sacrifice tail blood cultures were obtained. Autopsies were performed at once under sterile conditions, and cultures of the lung were made.

The death rate among 26 controls was 96 per cent and the average time of death 3.2 days. With the exception of two late deaths (5th and 7th days) all control animals died on or before the 4th day. Nine control rats were sacrificed during the first 48 hours. Of 10 animals treated 8 days and designated for 15th day sacrifice there was a death on the 8th and another on the 11th day. Among the 37 treated animals serially sacrificed (1-7 days) there was a single 5th day death.

A comparison of the pneumonia in the treated and untreated groups demonstrates that sulfanilamid altered the course and the extent of the induced pulmonary infection. This was apparent at 24 hours, where the lesion was limited in extent of consolidation, and showed smaller amounts of edema fluid. The treated animals showed at 24 hours a great reduction in the number of bacteria present, and in some, despite a well established fibrinopurulent pneumonia, organisms could not be found either in section or by culture. The control animals all showed a spreading fibrinopurulent pneumonia with myriads of bacteria. Pleural involvement occurred in 92 per cent of the control animals, and in 23 per cent of the treated. The incidence of pulmonary infarction, thrombosis and abscess formation was approximately 25 per cent in untreated animals, slightly less in treated. Lung and blood cultures of control rats were uniformly positive for pneumococci at death. A single positive blood culture was observed in the treated group—this from one of the three fatalities. Pneumococci were recovered from 30 per cent of the lung cultures

of the treated group, and these grew on blood agar as smooth, mucoid (virulent) colonies and gave a positive Neufeld reaction.

The striking finding in the microscopic picture of treated pneumonias 2 or more days old was the predominance of the mononuclear cell in the alveolar exudate, which at 24 hours was comprised of polymorphonuclear leukocytes. This change is correlative to the findings of Robertson and his co-workers in immunized dogs recovering from experimentally induced pneumonias. The appearance of the mononuclear cell is closely associated with the disappearance of bacteria; this is supported by the observations of Nungester and Gunn of the appearance of mononuclear cells and the disappearance of organisms from the lungs at 48 hours when dilute infecting doses of pneumococci were used.

Phagocytosis of bacteria was inconspicuous in all treated animals and appeared to play no significant part in the disappearance of bacteria. While no immunological studies have been made, antibody titrations in pneumococcal infections in man, dog and mouse suggest that the early disappearance of organisms in treated rats probably precedes the formation of antibodies. The mode of action of sulfanilamid would seem to be bacteriostatic and bactericidal in the light of this work.

### *Discussion*

(Dr. Herbert L. Reichle, Cleveland, Ohio.) Before the organisms disappeared, were there any observations made on the capsule in the treated animals?

(Dr. Theodore J. Curphey, Westbury, N. Y.) I would like to ask whether cultures of the lungs of the rats at postmortem showed organisms other than the pneumococcus injected. I did some experiments along these lines not so long ago, and felt that because of the presence of so many contaminating organisms, especially in the control group, that the picture did not give a fair comparison with the lesion found in pneumococcus pneumonia in human beings.

(Dr. Goldstein, closing.) As to the demonstration of capsules, one can only gather that by inference from the refractory zone about the pneumococcus. My attempts to demonstrate capsules by capsule stains in the tissues have not been successful. I am familiar with the work demonstrating the disappearance of capsules, but we cannot make any definite statements about this.

As to a variety of organisms in the cultures, with the technic we used, pure cultures were obtained in something like 99 per cent of cultures at autopsy, and this result obtained even if the animal died during the night and the autopsy was made the next morning.

STUDIES ON THE HISTOPATHOLOGICAL CHANGES PRODUCED IN RABBITS BY EXPERIMENTAL INOCULATION WITH THE HEMOLYTIC STREPTOCOCCUS, ITS NUCLEOPROTEIN AGGLUTINOGENIC FRACTION AND 9 PER CENT SAPONIN. PRELIMINARY REPORT. Lawrence W. Smith, Isabel M. Morgan (by invitation) and Stuart Mudd, Philadelphia, Pa.

*Abstract.* The experimental material consisted of a series of 65 rabbits inoculated variously with whole organisms, with chemical fractions of these organisms and with another known hemolytic agent (saponin) as a control. In most of the work hemolytic streptococci of Lancefield Group A were used, but in addition, the effect of organisms of Groups B, C and D on the animals

was also studied. In general, it may be stated that no changes either of a toxic or vascular nature were demonstrated in the normal controls; that either no recognizable, or at the most only minimal changes could be found in those animals inoculated with nucleoprotein agglutinin; that both toxic degenerative parenchymatous changes, and vascular lesions indistinguishable in kind from those reported by one of us (L. W. S.) in human fatal scarlet fever, occurred in these animals injected with whole streptococci, whether through the use of heat-killed organisms or by the infected subcutaneous blood clot method; that saponin as a hemolysin tended to produce severe degenerative parenchymatous visceral lesions; and finally, that similar toxic and vascular changes could be demonstrated in animals treated by injection of the partially purified lytic fractions. Similar changes differing only in being of lesser degree were noted in animals inoculated with the B, C and D types of hemolytic streptococci. From this material the hypothesis suggests itself that the nucleoprotein fraction might prove to be of value as an immunizing agent, in experimental infections in animals, thus avoiding the harmful effects of the toxic lytic fractions.

INTRACYSTIC PAPILOMA OF THE BREAST. Otto Saphir, Chicago, Ill.

*Abstract.* A histological investigation of 58 intracystic papillomas of the breast reveals three distinct varieties of these tumors: a fibrous type, a glandular type, and a papilloma consisting of cells which closely resemble the transitional epithelium of the urinary bladder, and hence, may be designated as the transitional cell type. The fibrous type consists of a stalk of connective tissue which often is very thin. Ramifications of the stalks may fuse with the production of pseudoglandular structures. This is the most common type of papilloma. Because of such pseudoglandular structures, this special subgroup of the fibrous variety may be designated as the pseudoglandular type. The glandular type is apparently formed by an extension of neighboring hyperplastic or adenomatous periductile acini into a duct or cyst, the epithelial cells of the duct enclosing the invaginated acini. Both the fibrous and the glandular types are benign tumors which do not recur. They often extend into the neighboring ducts but cannot be regarded as precancerous. The transitional cell type of papilloma morphologically is a benign tumor. However, it may recur after removal and some of the recurrent tumors may show morphological evidence of malignancy. It resembles the papilloma of the urinary bladder which, though morphologically benign, is sometimes classified as carcinoma Grade I principally because of recurrences which prove to be malignant. Intracystic papillomas of the breast are as a rule multiple. The multiplicity of these transitional cell papillomas may be explained on the basis of multiplicity of origin or by implantations of tumor cells in neighboring ducts. Such implants do not necessarily indicate malignancy, but may be compared to certain benign ovarian tumors which occasionally produce implantations on the peritoneal surface.

#### *Discussion*

(Dr. Carl V. Weller, Ann Arbor, Mich.) I should like to ask if Dr. Saphir finds the basal cell layer of the breast concerned in any of these three types of papilloma.



(Dr. Saphir.) It is usually the upper layers of the cells lining the duct structures, rather than the deeper layer, which are concerned in papilloma.

**SPLENIC NEOPLASMS.** Samuel A. Goldberg, Newark, N. J.

*Abstract.* Primary neoplasms of the spleen are quite rare. A review of the literature shows mostly reports of individual cases. The various types of primary neoplasms reported in order of their occurrence are: lymphoblastoma, lymphangioma, hemangioma, endothelioma, and rarely fibroma and fibrosarcoma. Some of the cases of lymphosarcoma reported in the earlier works, according to Klemperer, should be discounted as they probably belong in the group of lymphadenoses or atypical Hodgkin's disease.

The angiomas range from simple localized or widely diffuse telangiectases to more or less encapsulated nodules, and finally to highly malignant endotheliomatous tumors with metastases.

Metastatic neoplasms of the spleen are also seldom encountered. This is not in keeping with the nature of the organ since embolic phenomena are fairly common in the spleen. As the spleen is both a vascular and a lymphatic organ, metastatic neoplasms should be more commonly seen. The paucity of such metastases cannot be explained on an entirely morphological basis, such as the limitation of lymphatics to the subcapsular region, the sharp angle of origin of the splenic artery, or the effect of splenic pulsation in preventing the lodgement of tumor cells. The explanation lies more likely in the fact that the spleen offers a poor soil for the growth of neoplastic cells. Lubarsch first pointed out that in order for cells to establish themselves in an organ they must overcome the resistance of the organ, and several generations of cells are destroyed before they can adapt themselves and are able to proliferate. Fichera speaks of an oncological ferment which hinders the proliferation of tumor cells. Krumbhaar states that the occurrence of fibrous nodules in the spleens of cancer patients may be further evidence of the antagonism of splenic tissue to malignant tumors. McNee reported 2 cases with development of epithelial tumors several years after an enlarged spleen had been removed. He ascribed the formation of these tumors to the removal of the factor in the spleen which hindered the growth of epithelial neoplasms.

Experimentally several investigators have found that splenic pulp mixed with transplanted tumor cells inhibited or retarded the growth of the tumors.

This report includes 3 primary and 9 secondary neoplasms of the spleen collected from 540 autopsies, 93 of which showed malignant tumors. This gives an incidence of 9.7 per cent of secondary malignant splenic neoplasms. Of this number, 5.37 per cent were gross nodular tumors, 2.2 per cent were microscopic metastases, and 2.2 per cent showed invasion by contiguity into the splenic pulp.

The neoplasms primary in the spleen were 1 hemangioma cavernosum and 2 lymphangiomas.

### *Discussion*

(Dr. E. T. Bell, Minneapolis, Minn.) I want to comment on the statement that the spleen is an unfavorable site for the growth of metastatic tumors. Someone about a year or so ago showed that the kidney has metastases less frequently than the spleen. The combined weight of the kidneys is a great deal more than the weight of the spleen, and yet they have fewer metastases

than the spleen. Our own autopsy records show the same thing. It is also true that the heart has metastases less frequently than the spleen, so if the spleen has some anticarcinogenic substance in it, the kidneys should have more.

(Dr. Howard T. Karsner, Cleveland, Ohio.) I am in full agreement with what Dr. Bell says. I think the paper justifies some comment on the use of the term neoplasm. To my mind the inclusion of the so-called hemangioma and lymphangioma, or mixtures of these, as true neoplasms, appears to be unjustified. This view naturally involves consideration of the place of the hamartomas.

(Dr. Kornel Terplan, Buffalo, N. Y.) I should like to stress the occurrence of the microscopic type of metastatic carcinoma in the spleen. I remember a few instances in our postmortem material of the last years in which the spleen was of normal size or even slightly atrophic, with no gross evidence of metastatic tumor. The site of the primary carcinoma was the stomach or the lung. Microscopically the spleen was found to be studded with carcinoma cells; they did not form nodules but had grown diffusely throughout the sinusoids. In 1 of these cases the liver showed a similar type of metastatic carcinomatosis. It was small and anemic. There was no gross evidence of carcinomatous nodules. Histologically, however, the capillary sinusoids showed most diffuse infiltration with carcinoma cells. From such surprising experiences I feel that microscopic metastasis to the spleen is apparently more frequent than we were inclined to expect.

(Dr. Goldberg, closing.) In regard to Dr. Karsner's remark about the question whether lymphangiomatous or hemangiomatous tumors are really neoplasms, according to the classification of Fowler they are supposed to be neoplastic lesions. The fact that they are nodular and have a stroma, according to the literature, is an indication that they are neoplastic. Some of these lesions may be simply telangiectatic, as I pointed out.

The fact that in 93 cases of malignant neoplasms there were 9 cases of metastases in the spleen certainly indicates that they are not as rare as the literature would have us think.

#### CARCINOMA OF THE LUNG. AN ANALYSIS OF SEVENTY-FOUR AUTOPSIES.

Rigney D'Aunoy, Bjarne Pearson (by invitation) and Béla Halpert, New Orleans, La.

*Abstract.* Seventy-four cases of primary carcinoma of the lung were encountered in 6623 autopsies on individuals over 1 year of age. Males and females were represented in the proportion 11:1. The age range was from 21 to 75 years. The average duration of illness was 5 months. Thirteen patients died in the 5th, 33 in the 6th, and 19 in the 7th decade of life.

In almost half of the cases the primary growth was located in one bronchus or the other.

Thirty-seven of the 74 cases were squamous cell, 21 were reserve cell, and 16 were columnar cell carcinoma.

#### *Discussion*

(Dr. Emmerich von Haam, Columbus, Ohio.) I should like to ask if there is a difference in the distribution of metastases in the 3 carcinomas.

(Dr. H. Gideon Wells, Chicago, Ill.) How large a proportion of this

great incidence of carcinoma of the lung may be attributed to the greater zeal in securing autopsies in cases of this class because the diagnostic problem excites interest on the part of the clinicians? It seems to me that has modified our statistics a great deal in institutions where there is not 100 per cent of autopsies, and I do not know of any such institutions.

(Dr. Howard T. Karsner, Cleveland, Ohio.) In another large city hospital the number of bronchogenic carcinomas disclosed at autopsy exceeded that of any other form of carcinoma, and I think we should refer to figures of this sort as representing the autopsy room population rather than the living population.

(Dr. James Ewing, New York City.) I should like to know if the authors found any evidence of incidence of the relatively benign papillary adenocarcinoma of the bronchi described by Crawford of Philadelphia. I would also like to ask if they draw any conclusions as to whether there are tumors derived from the lining cells of the alveoli.

(Dr. Wiley D. Forbus, Durham, N. C.) There is an interesting bronchial tumor which Dr. Halpert has not mentioned. It is one which has the gross characteristics of a colloid carcinoma. I use the term carcinoma with some hesitation because the tumor is one which does not invade very widely but acts rather like a benign tumor. Histologically, however, it is definitely invasive. It would be interesting to know where Dr. Halpert puts this tumor in his classification.

(Dr. Harry C. Schmeisser, Memphis, Tenn.) Drs. W. Likely Simpson and Robert M. Moore, from our departments of otolaryngology and pathology, published a case of primary colloid adenocarcinoma of the lower third of the trachea, which was shown to take origin from the epithelium of the mucous glands below the surface.

(Dr. Halpert, closing.) In reply to Dr. von Haam's question, there is no significant difference in the metastases of one type or the other type of carcinoma. This series has been too small to draw any conclusions in that direction.

In reply to Dr. Wells, of course this represents autopsy material. However, in this particular series perhaps it does not represent increased zeal in securing autopsies. These were patients who quite frequently died without a correct diagnosis, and the finding at autopsy of a carcinoma of the lung was accidental.

In answer to Dr. Karsner's remark, perhaps his hospital deals with the same kind of clientele as ours, and that may be the reason that he can put one over on the Charity Hospital as far as the number of autopsies of carcinoma of the lung is concerned.

As to Dr. Ewing's question, this is a study of 74 carcinomas seen at autopsy, and since I have not come across the kind he mentioned, I could not say anything about it. In reply to his question as to whether we found evidence of origination of carcinoma from the alveolar lining cells, I have not found any of those. I feel quite confident that the lining cells of the alveoli of the lung rarely, if ever, give rise to malignant neoplasms. The parent cells of all carcinomas of the lung, I believe, are the reserve cells.

In regard to the comments of Dr. Forbus and Dr. Schmeisser, I would regard both of those tumors as columnar cell carcinomas, and they of course can produce mucus. They are derived from reserve cells which differentiate into the cell form which they normally produce.

STROMAL TUMORS OF THE CHOROID PLEXUS. Amour F. Liber and James R. Lisa, New York City.

*Abstract.* The choroid plexus is made up of two kinds of tissues, the epithelium and the stroma, which are distinct embryologically and anatomically. The stroma is an infolding of the leptomeninx. Tumors derived from the stroma are indeed homologues of those of the meninges. Meningioma is the most common neoplasm of the stroma. Others are sarcoma, angioma, chondroma, lipoma, myxoma, teratoma and deposits of calcium, iron or cholesterol with phagocytic and fibrotic reactions. In man, meningioma, sarcoma and angioma are the only neoplasms that reach a large enough size to be of clinical importance.

A typical case of meningioma of the choroid plexus of the left lateral ventricle has been studied. The tumor filled and distended the entire body and parts of the frontal and occipital horns of the ventricle. It was attached to the dorsal aspect of the glomus by a short stalk of fibrous tissue and blood vessels covered by choroid tufts. Microscopically elongated bundles of syncytial bands containing fibroglia and fusiform vesiculous nuclei were lined up end to end in long straight rows. Collagenous and reticulum fibers coursed between the cytoplasmic bands. In some places fine reticulum fibers came into close contact with the cells and seemed to penetrate them. No elastic fibers were found. Occasionally there were cellular whorls which contained no collagen and only rare reticulum fibers. No mitoses were seen. Blood vessels were scarce and thin walled. There were no calcospherites. The tumor was surrounded by a thin hyalinized capsule, which was partially adherent to the ventricular wall. The ependyma persisted in some of the adherent areas but was absent in others.

The remaining cases were autopsy discoveries. Two cases revealed large, cystic calcareous deposits surrounded by dense fibrotic scar tissue. A small fibrolipoma was found in the plexus of the lateral ventricle. The fibroblastic portion of the growth was sharply demarcated from the adipose tissue and presented the features seen in fibrosed arachnoidal granulations. Adipose tissue is not a normal constituent of the choroid plexus stroma. It is a question whether it arose as a heteroplasia or as a transformation *in situ* of leptomeningeal tissue. Reports of only 4 previous cases of lipoma of the choroid plexus could be found. In 3 cases of congenital hydrocephalus with spina bifida in infants, narrow fibrovascular stalks projected dorsalward from the glomus of the choroid plexus bilaterally. The tip of the stalk was thickened to form a terminal nodule consisting of hyperplastic cellular and collagenous stroma which, similar to that seen in fibrolipoma, had a structure suggestive of arachnoidal granulations. In 1 case calcospherites were present. In a case of unilateral hydrocephalus, associated with an anomalous arrangement of the large veins of the ipsilateral cerebral hemisphere, a long stalk projected dorsalward from the glomus in the dilated ventricle only. There was no terminal nodule.

In all of these cases the growth occurred in or was attached to the glomus of the choroid plexus. The hypothesis is formulated that hydrocephalus, the formation of a stalk and the hyperplastic nodules are steps in the development of meningiomas. In this respect, as in their structure, the hyperplastic glomic nodules would seem to be analogous to the arachnoidal granulations.

*Discussion*

(Dr. Ralph D. Lillie, Washington, D. C.) In regard to the possible histogenesis of this fibrolipoma, if you call it that, of the choroid plexus, I might remark that fat is not too infrequently a component of the stroma of the choroid plexus in some of the laboratory animals I have been studying in recent years.

(Dr. Liber.) Some varieties of lipid have been reported in a number of animals. I am familiar with that in horses. It has been found by veterinarians that 8 per cent of old horses (I do not know what age they call old in a horse) have xanthomas, sometimes called cholesteatoma vasculosa. From the description given by different pathologists who have studied these growths, they are not lipomas but xanthomas with doubly refractile lipids and cholesterol crystals, foreign body giant cells, and as a rule an intense inflammatory reaction. This is in the horse.

(Dr. Lillie.) That is not the type of thing I am referring to. It is apparently ordinary body fat.

(Dr. Liber.) In what animals?

(Dr. Lillie.) In guinea pigs in particular.

**METASTATIC TUMORS OF THE MYOCARDIUM.** Gorton Ritchie, Madison, Wis.

*Abstract.* Fifteen cases of tumor metastases in the myocardium have been studied. Thirteen different types of primary tumor were found. Carcinoma of the lung occurred 3 times; there was 1 each of the following tumors: rhabdomyosarcoma of the kidney, carcinoma of the esophagus, mesothelioma of the pleura, myogenic sarcoma of the bladder, melanosarcoma, carcinoma of the rectum, carcinoma of the fundus uteri, carcinoma of the head of the pancreas, and lymphosarcoma (primary source undetermined). The malignant cells were carried to the heart through the blood stream in a majority of the cases, but the spread in the cardiac wall was frequently by way of the lymphatics. In 1 case (carcinoma of the esophagus) the only "remote" metastasis was to the heart muscle by way of the blood stream; in all of the others the metastases were fairly widely distributed (except 1 case of sarcoma of the lung in which the invasion of the heart was by direct extension). Although involvement of the heart was quite extensive in some cases, in no instance was the diagnosis made clinically.

In addition, 23 metastatic tumors of the pericardium were found, representing 11 different types of primary tumor.

*Discussion*

(Dr. William Boyd, Toronto, Canada.) May I ask Dr. Ritchie where the metastases were in the case which he showed as being one of sarcoma? The evidence which he adduced in support of that diagnosis was that the tumor was massive in character and that the reticulum stain suggested sarcoma. Certainly a massive growth in no way eliminates carcinoma. I think Dr. Karsner will agree with me when I say that one has to be very careful in drawing conclusions from the use of the reticulum stain. I feel in these cases the natural history of the disease, in other words the distribution and character of the metastases, is of the greatest value in deciding between carcinoma and sarcoma, and I think the speaker would have to give us very convincing

evidence to make us accept this tumor as a sarcoma, judging from the fleeting glance I had of it on the screen.

(Dr. Carl V. Weller, Ann Arbor, Mich.) Within the last 3 weeks we have had a case similar to the one reported, a squamous cell carcinoma of the esophagus with metastases to the apical portion of the left ventricle without pericardial adhesions and without any traceable extension by contiguity in the lymphatics.

(Dr. Ritchie, closing.) I was almost afraid to include this lantern slide for fear it would lead to such a controversy. The metastases were merely local in this case and were not widespread throughout the body. It is on the evidence of the definite small spindle cells within the tumor and the very apparent connection of the reticulum with these cells that I have proffered this diagnosis.

CONCEPTS OF A NEW CLASSIFICATION OF OVARIAN TUMORS. Walter Schiller  
(by invitation), Chicago, Ill.

*Abstract.* Several attempts have been made during the course of the last few years to suggest a better and more satisfactory classification of ovarian tumors than that usually found in the textbooks of pathology or gynecology. Counseller and Broders, Cornill, and Leroux, Levret and Weinroth suggested classifications more or less founded on anatomical or clinical facts. These classifications serve very well for practical and clinical purposes, but do not satisfy the aim of the pathologist to have a system founded on the histogenetic explanation of the various newgrowths. Recent experiences and investigations justify trial of a histogenetic system. First the cysts which are physiological cavities dilated by pathological secretion or retention are separated from the true neoplasms. These cysts are classified according to the cavities from which they develop as follicular cysts arising from follicles, corpus luteum cysts arising from corpus luteum, and corpus atreticum cysts usually called lutein cysts. The neoplasms may be split into two large groups — those developing from true ovarian tissue or ovariogenetic, and those developing from tissue which is not found in the normal ovary, or heterotopic tumors. The ovariogenetic tumors include the fibroma from the fibrous tissue and the granulosa cell tumor from the granulosa. The heterotopic tumors have as the first subgroup tumors developing by protoplasmic differentiation from the surface of the epithelium. They duplicate either tubular epithelium and form serous cystomas, or the endometrium, forming endometriomas, or the cervical mucous membrane, forming pseudomucinous cystomas. The second group is represented by error in sex chromosomes occurring in fetal remnants of the mesenchyma. When the sex differentiation is male they form arrhenoblastomas; when the differentiation is neither male nor female, but neutral, they form disgerminomas. The third group contains the tumors developing from misplaced blastomeres. This group includes the mature teratoma represented by the dermoid and the immature teratoma or embryoma. The fourth group develops from misplaced neighboring tissue and is represented by hypernephroma, ganglioneuroma, Brenner tumor (epithelium of uropoietic organ), and mesonephroma. The fifth group contains tumors developing by an actual transference of extraovarian tissue to the ovary during life. This group can be divided into two subgroups: the metastatic tumors (for instance, the Krukenberg tumor) and those particular endometriomas

originating by implantation of endometrium on the surface of the ovary, according to Sampson.

### *Discussion*

(Dr. James Ewing, New York City.) Dr. Schiller proposes to wipe the slate clean and start over again classifying ovarian tumors on a histogenetic basis. Such an effort is to be commended, if it can be made successful. The majority of ovarian tumors are cystadenomas which are readily identified, although their exact histogenesis may still be somewhat uncertain. The difficulty arises when dealing with a smaller group of malignant tumors of which the structure varies extremely and the histogenesis is highly obscure. In this latter group most pathologists recognize some types which are rather specific and easily recognized, such as the Brenner tumor, the seminoma, and malignant variants of the dermoid, but most of these cellular tumors present a very varied and often mixed structure which it has been difficult to interpret. In recent years the studies of Robert Meyer and many others have brought new data into this field by showing that striking changes in the secondary sex characteristics occur in certain of these tumors, and they have undertaken the classification of these growths in the light of these hormonal effects. New interpretations of the embryology of the ovary have also been introduced as guides in the classification. Varangot has published recently a very excellent monograph covering all these new data and has presented a classification based thereon. When one reads the new contributions one may obtain the impression that it is now comparatively easy to identify the cellular malignant tumors, especially when clinical data relating to the sex changes and the endometrium are available. However, after arming myself with much of this new information I found to my great disappointment that the atypical malignant tumors were about as difficult to recognize as ever. The structure varies extremely, the sex changes are not reliable, and the embryology of the ovary is highly obscure. Moulouguet has said that the embryology of the ovary offers no reliable basis for the classification of ovarian tumors. The subject of intersexuality proves to be even more complex than that of ovarian tumors. Recently, in reviewing a series of complex malignant ovarian tumors and observing the diagnoses submitted by pathologists who have enjoyed some reputation in this field, I came to the conclusion that the final diagnosis adopted is largely a matter of arbitrary decision on the part of the observer. No doubt many of the so-called granulosa cell tumors with feminizing properties are recognizable with certainty, but many others are not. Arrhenoma presents a very mixed and variable structure of spindle and polyhedral cells, and masculinizing effects are not limited to tumors derived from male elements. Theca cell tumors and so-called luteinizing forms of granulosa cell tumors must be identified on very uncertain grounds, such as fat droplets. The specific structures assigned to these various tumors by many writers obviously overlap one another.

Under such circumstances I cannot think that adequate knowledge is yet available on which to establish the actual nature of many malignant ovarian tumors. Dr. Schiller's feeling that we must revert mainly to a morphological basis and endeavor to establish the histogenesis of these tumors seems therefore to have considerable support, but I fear that here again the difficulties are quite formidable. I can report the observation of 1 minute ovarian carcinoma, 3 mm. in diameter, submitted recently by a Brooklyn physician.

Unfortunately it had a very mixed structure, some acini resembling Pick's testicular adenoma, others suggesting a granulosa cell tumor, with a few strands of spindle cells like arrhenoma.

(Dr. Harry C. Schneisser, Memphis, Tenn.) I recently published in collaboration with Dr. W. A. D. Anderson the report of a ganglioneuroma of the ovary which could easily be diagnosed from its histology. We considered its origin to be from the groups of sympathetic ganglion cells which occur normally in the medulla of the ovary near the hilus. I wish to ask Dr. Schiller where he would place this tumor in his classification.

(Dr. Schiller, closing.) The ganglioneuroma falls into the group of tumors developing from neighboring tissue misplaced in the later period of fetal life.

Concerning the papers of the French authors, especially Varangot, I have studied these papers but have the feeling that some of these men make the mistake of relying too much on physiology and too little on morphology. Whenever it is possible we should rely on the morphological, and if possible on the histogenetic-embryological changes. When one starts to classify tumors according to the products they furnish, one may have to place heterogeneous elements in one group. There would be a group called virilizing tumors, which would include hypernephroma and arrhenoblastoma, although they are morphologically and histogenetically different.

In no other field of pathology do we make our classification according to the physiological effect of the tissue, for every physiologist knows that different tissues may produce the same secretions. Whenever it is possible to furnish a classification, it is preferable to make a tentative classification on a morphological basis rather than to accept physiological classifications which may include errors.

CARCINOMA OF THE KIDNEY IN A COLONY OF RHESUS MONKEYS. Herbert L. Ratcliffe, Philadelphia, Pa. (Presented by Dr. Herbert Fox.)

*Abstract.* Because of the relative infrequency of neoplasms in infrahuman primates, it may be of interest to record the occurrence of carcinoma of the kidney in 4 rhesus monkeys (*Macaca mulatta*). Of further interest is the fact that these animals were members of a family group, the male parent and 3 offspring, a female and 2 males, being involved.

In each case the tumor developed in the left kidney, expanding the capsule and replacing the parenchyma. In only 1 animal were secondary tumors found.

The average age of these animals was 181.5 months, approximately 10 times the average of the group.

#### Discussion

(Dr. Otto Saphir, Chicago, Ill.) I should like to ask whether or not some of the tumors were bright yellow and contained fat, in other words did some of these tumors resemble the hypernephroma type of carcinoma or not?

(Dr. Fox.) Tumors Nos. 1 and 4 had a moderate number of so-called foamy cells; they were not examined for double refractile lipid bodies. Tumor No. 3 was normally opaque. It did not contain foamy cells.



THE PATHOLOGY OF MAMMARY CARCINOMA OF THE RABBIT. H. S. N. GREENE,  
Princeton, N. J.

*Abstract.* One phase of an extensive constitutional study under progress in our laboratory has been an investigation of neoplasia in a large colony of rabbits, and as a result of routine clinical and pathological examination a considerable number of spontaneous tumors have been found. Two distinct morphological types of mammary carcinoma which differ in mode of development and biological characteristics have been observed, and our type is further distinguished by a characteristic antecedent breast history. A history of cystic disease preceded the development of tumors in 21 cases, while in 4 cases no abnormal antecedent changes in the breast were noted. Tumors in cystic breasts arose as papillomas from the epithelial lining of dilated duct and cyst walls and with continued growth formed multiple radicles which anastomosed with the production of numerous acinar-like structures. In such cases cystic disease, non-invasive neoplasia and cancer occurred as succeeding events in the breast and apparently formed parts of a continuous disease process. The second class of tumors, on the other hand, originated in normal breasts and arose from a proliferation of true acini.

Pronounced pathological changes were found in organs of the endocrine system in all tumor-bearing animals and were present from the earliest stages of tumor development. On the contrary, such changes were not found in animals bearing the transplanted tumors, although more than 150 were examined after periods of growth ranging up to 6 months. Histologically the alterations were identical with those observed in animals subjected to long continued treatment with oestrone, and because of this it is suggested that the spontaneous tumors represent a natural analogue to the experimental induction of neoplasia with such substances.

*Discussion*

(Dr. Arthur W. Wright, Albany, N. Y.) I am very much interested in the colony of rabbits Dr. Greene has described. We have in our laboratory a strain of rats in which mammary tumors are appearing fairly abundantly. In all cases but one they have been benign fibroadenomas. The exception was a metastasizing adenocarcinoma. In these animals we have found changes in the pituitary and adrenal glands. The latter organs have not yet been thoroughly studied but the pituitaries were increased in size and often showed small adenomas which were primarily of the chromophobe type. In general the chromophilic cells were diminished in number, particularly the eosinophils. We have been slowly inbreeding these animals and have been attempting to obtain high tumor lines. At the moment the work has not gone far enough to know whether we shall be successful in this attempt. I should like to ask Dr. Greene the incidence of spontaneous mammary tumors in his colony so far.

(Dr. Emmerich von Haam, Columbus, Ohio.) I should like to ask Dr. Greene if he has made any determination of the oestrin content in the tissues of these animals.

(Dr. Clarence M. Lightner, New York City.) Did any of the tumors occur in males?

(Dr. Greene, closing.) In answer to the last question, all the tumors were

in females. There were 2 males in the tumor line, however, which showed adenomyosarcoma of the kidney.

We have no funds or facilities for determining the oestrin content in the tissues, I am sorry to say.

Inasmuch as the tumors occur in family lines and not in the general population, an analysis of the incidence based on the colony as a whole would be without significance and has not been attempted. Relatively few animals of the line have been held to a tumor age because of inadequate facilities. At the present time approximately 100 have been under observation for 3 or more years and of these 25 developed tumors.

THE SKINS OF GUINEA PIGS AFTER FIVE YEARS OF DAILY APPLICATIONS OF 1:2:5:6 DIBENZANTHRACENE. Roland S. Aronson (by invitation), Philadelphia, Pa.

*Abstract not received.*

### *Discussion*

(Dr. Hugh G. Grady, Cambridge, Mass.) May I ask Dr. Aronson if he has attempted any subcutaneous injections of the carcinogenic agent in guinea pigs? I ask that question because Dr. Shear in collaboration with Drs. Elliot and Howe has succeeded in inducing subcutaneous sarcoma in the guinea pig by implantation of benzpyrene crystals.

(Dr. Stanley P. Reimann, Philadelphia, Pa.) I have no remarks to make except that the dibenzanthracene which was used was carcinogenic in mice because we used material from the same bottle and it induced carcinoma in them.

(Dr. Aronson, closing.) My answer to Dr. Grady's question is no.

HISTOLOGICAL STUDY OF THE DEVELOPMENT OF PULMONARY TUMORS INDUCED BY 1:2:5:6-DIBENZANTHRACENE AND METHYLCHOLANTHRENE IN STRAIN A MICE. Hugh G. Grady (by invitation) and Harold L. Stewart, Cambridge, Mass.

*Abstract.* Based on the observation that tumors can be induced in the lungs of mice treated with dibenzanthracene or methylcholanthrene, an experiment was devised whereby a study could be made of serial histological sections of tissue from the lungs of mice in the period during which the pulmonary tumors were developing.

Two hundred Strain A mice, 2½ to 3 months of age and equally divided as to sex, were used. One hundred mice received 0.8 mg. of 1:2:5:6-dibenzanthracene in 0.8 cc. of lard subcutaneously; 60 mice received 1.6 mg. of methylcholanthrene in 0.4 cc. of lard subcutaneously; and 40 mice received 0.8 cc. of plain lard subcutaneously and served as controls. Animals which developed tumors at the site of injection, or which died as a result of hemorrhage, infection, or any other cause, were not included in the study. The final effective number consisted of 130 mice injected with the carcinogens and 30 control animals. The animals were sacrificed daily (except on Sundays and holidays) over a period of 3 months, 98 being killed between the 26th and 60th days. In all animals the lungs were fixed per tracheam in Zenker's fluid, and the entire right lower lobe was sectioned in series. The remaining

lung tissue was embedded in paraffin and kept in reserve. Sections were routinely stained with eosin-methylene blue and Masson's trichrome technic. Foot's method for reticulum and phosphotungstic acid hematoxylin were also used on occasion.

The earliest recognizable pulmonary tumor was found 32 days after injection of methylcholanthrene and 36 days after injection of 1:2:5:6-dibenzanthracene. No difference in the character of the tumors produced by the two hydrocarbons was observed. From the 40th day onward, tumors were found with increasing frequency. All tumors examined appeared to be adenomatous growths and were histologically similar to those previously described as induced tumors; they also resembled closely the spontaneous lung tumors of mice. Few, if any, were connected with the bronchial epithelium at any point. In most instances the tumors were of multicentric origin and located close to the pleura or in actual contact with it. Immediately preceding the development of tumors and accompanying the early stages of their formation was a notable proliferation of large mononuclear cells from the alveolar walls. These cells at first varied in shape, depending apparently on their relation to the alveolar walls. Frequently they would form columns of varying length partly or completely lining the alveolar space, or they might coalesce to form small groups. These groups might project into the alveolar lumen or occur within or on the septal wall. The adenomatous nodules appeared to develop through a combination of these processes, but when developed they were remarkably uniform in appearance. The recognizable tumor was composed of more or less closely packed columns of cuboidal or low columnar cells with relatively large nuclei. The nuclei were either deeply staining or vesicular and few mitotic figures were seen. The cytoplasm was usually slightly acidophilic and finely granular and occasionally contained small particles of phagocytosed material.

### *Discussion*

(Dr. H. Gideon Wells, Chicago, Ill.) It is interesting to me to see these early changes in induced tumors because in looking over many hundreds of spontaneous tumors among Dr. Slye's mice, all these pictures are there, and we have been impressed with the apparent evidence that these tumors in mice represent, as near as we can tell from looking at them, an epithelial tumor arising in the alveolar lining. I have seen tumors spontaneously arising in which I thought the tumor came from the bronchus. I was interested in Dr. Grady's statement, but he did not make it complete, on the character of the changes that take place in the transplants, and the thing which has impressed us is this: when these tumors produce metastases, as they often do, many of the metastases look like sarcomas, although the primary tumors do not look like sarcoma in most cases. The lung would be filled up with what looked like an epithelial growth and the metastases looked exactly like a sarcoma.

(Dr. Grady, closing.) That is an interesting question which makes another paper in itself. We have now seen close to 50 of these tumors which have been carried in serial transplants in which, along about the 3rd or 4th transplant, the tumor changes from what is apparently a typical epithelial growth to one which I do not think any one would quarrel with as being a spindle cell sarcoma. It is a very annoying and difficult phenomenon to explain. Incidentally Andervont has reported that finding. It should be noted that while most of the transplants are in induced tumors, Andervont has recently

observed this sarcomatous change in the transplant of spontaneous tumors which is of significance. That is why we hesitate to call these growths purely epithelial, even though they certainly look like it.

THE EFFECT OF THYROID FEEDING ON TUMOR GROWTH IN THYROPARATHYROIDECTOMIZED RATS. R. L. Ferguson, R. D. Templeton and Mary C. Patras (by invitation), and F. A. McJunkin, Chicago, Ill.

*Abstract.* In this experiment 135 thyroparathyroidectomized albino rats were used. Litter mates of the same sex and as near the same weight as possible were divided into two groups. Group 1 received Fox Chow only. Group 2 received Fox Chow which contained 0.02 per cent desiccated thyroid. The animals were kept on their respective diets for 150 days, at which time two small pieces of tumor tissue (rat spindle cell sarcoma) were placed beneath the skin through a dorsal incision at the level of the first lumbar vertebra.

The animals were weighed weekly and tumor measurements were obtained at that time. The thyroid-fed animals lived on an average of 39 days, while the animals on Fox Chow lived only 35 days.

Desiccated thyroid was observed to have a favorable effect on tumor growth. The importance of this observation, however, is diminished when one considers the stimulating effect of thyroid on the host.

#### *Discussion*

(Dr. Harry S. N. Greene, Princeton, N. J.) I should like to ask if these observations were limited to young animals. I ask this because in our experiments the action of thyroxin appears to be related to the age of the tumor-bearing animal. The administration of thyroxin to young rabbits bearing a transplanted uterine tumor results in rapid growth. In mature animals, on the other hand, this procedure results first in slow growth in a histologically more highly differentiated pattern, and finally in regression.

(Dr. Ferguson, closing.) For thyroparathyroidectomy we used young animals which were litter mates of about the same age and about the same weight. We obtained no regression; the animals died around 36 to 39 days after tumor transplants. The sex made a little difference. The male animals were slightly heavier than the females.

THE EFFECT OF COMBINED ANDROGEN AND ESTROGEN ON THE PROSTATE.  
Robert A. Moore and (by invitation) Allister McLellan, New York City.

*Abstract.* In a previous study it has been shown that the injection of estrogens in men induces certain characteristic histological changes in the prostate. These changes are essentially an exaggeration of the metaplasia and lymphoid hyperplasia found in all cases of benign hypertrophy.

In animals the simultaneous injection of androgens with estrogens neutralizes the effects of the estrogens. In women the ratio to give complete neutralization is about 1 unit weight of estrogen and 50 units weight of androgen.

A histological study of the prostate from men who received varying combinations of estradiol benzoate and testosterone propionate shows that the neutralizing ratio is also about 1:50. From this it is concluded that the tissues of the two sexes are not selectively sensitive to the homologous sex hormone.

MALABSORPTION OF FAT (INTESTINAL LIPODYSTROPHY OF WHIPPLE). H. L. Reinhart and (by invitation) S. J. Wilson, Columbus, Ohio.

*Abstract.* In 1907 Whipple suggested the term "intestinal lipodystrophy" for a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues. In 1923 Blumgart reported 3 somewhat similar cases as "malabsorption of fat," and in 1936 Jarcho described a 5th similar case as "steatorrhea with unusual intestinal lesions" and reviewed the previous cases. A 6th case presenting the characteristic anatomical features and a clinical picture similar to that described by Whipple and Jarcho as anemia, progressive emaciation, chylous ascites, deposits of fat in the small intestines, mesenteric and retroperitoneal lymph nodes, and a relatively normal pancreas is described. In addition the patient presented the blood picture of a benign lymphocytosis suggestive of an atypical lymphoid leukemia. Although intestinal lipodystrophy is related to the xanthomatous diseases and steatorrhea in the anatomical and clinical manifestations of a disturbed lipid metabolism, it is fundamentally different from each of these conditions. The term intestinal lipodystrophy offers less objections and has more points in its favor than others suggested, particularly until more is known concerning the cause and genesis of the lesions.

### *Discussion*

(Dr. J. D. Kirshbaum, Chicago, Ill.) Was the bone marrow studied in this case, and was the diagnosis of aleukemic lymphadenosis considered here, in view of the large liver and spleen?

(Dr. William L. Robinson, Toronto, Canada.) Some years ago I had an opportunity to study the glands from a case in which the thoracic duct was tied off for experimental purposes. This produced a tremendous enlargement of the glands with dilatation of the sinusoids of the lymph spaces, but it showed no picture such as we have seen here — just simple dilatation.

(Dr. Ellis Kellert, Schenectady, N. Y.) Did you find inflammatory changes at the root of the mesentery which might explain the obstruction? Some years ago in a similar case we encountered a definite old inflammatory mass in the mesentery, and the pictures here recalled that case to me, for the intestinal changes are identical. I believe that recently there have been 1 or 2 cases reported under the diagnosis of chyladenectasis mesenterica. There may be other cases listed under that name.

(Dr. Henry W. Ferris, Ithaca, N. Y.) Some months ago I did an autopsy on an individual in whom there were lesions somewhat similar to those reported here. The mesenteric lymph nodes were yellow and almost completely replaced by fatty tissue, and fat was found in the mucosa of the intestine. In addition there was a widespread inflammatory reaction on all the serous surfaces, which might be called a polyserositis; it was on the pericardium, the peritoneum and the pleura, and there was evidence of organization of old fibrinous deposits. That might bear some relation to the fatty deposits mentioned here. I wonder whether anything has been found in the literature which would show an association between these conditions.

(Dr. Goodman, Rhode Island.) Will Dr. Reinhart describe the findings in the spleen and liver?

(Dr. Alfred Plaut, New York City.) I would like to know whether the lymph nodes were distinctly yellowish or brownish. Only the other day while

going over the organs of a case of multiple myeloma I was struck by the brownish color of one parapancreatic lymph node. This lymph node gave the histological picture just shown by Dr. Reinhart. In addition, small foci of fatty material partly surrounded by giant cells were found in the spleen.

(Dr. Reinhart, closing.) In answer to Dr. Kirshbaum's question, no study of the bone marrow was made. The hematological study of the case was conducted by Drs. Doan and Wiseman, and a biopsy of a superficial lymph node revealed no evidence on which to base a definite diagnosis of aleukemic leukemia. Benign leukemic lymphocytosis was considered.

In compliance with Dr. Goodman's request, I wish to say that the spleen was enlarged and there was present passive congestion with multiple infarcts. There was no evidence of focal lipid deposits in the spleen of the type encountered in some of the xanthomatous diseases. The liver was enlarged and there was a diffuse fine granularity of the surface; there was a moderate proliferation of the bile ducts, a moderate amount of portal fibrosis, and a rather marked lymphocytic infiltration of portal distribution which is encountered in portal cirrhosis. There was very little residual evidence of any preceding fatty metamorphosis in the liver.

The autopsy described by Dr. Ferris seems to have presented a similar if not an identical picture. The tissue reactions are very complicated and numerous factors undoubtedly play a part in their genesis. There appears to be some evidence that this condition is related to the leukemias, particularly monocytic leukemia, and possibly lymphoid leukemia and reticuloendotheliosis.

In answer to Dr. Plaut, the color of the lymph nodes was predominantly of a creamy rather than a brown color. There were small foci of a deeper yellow color which were more nearly orange than brown. In addition there were foci of recent hemorrhage.

In regard to Dr. Kellert's question, there were no inflammatory nodes at the root of the mesentery; the nodes at the root of the mesentery and around the pancreas were similar in character to the retroperitoneal lymph nodes. The retroperitoneal nodes were larger than those in the mesentery and this was the only difference noted. Certainly stasis of this lipid material is present, and it probably is a functional factor, but evidence of a primary blockage of the lymph channels was not obtained. I might say that numerous cases have been reported in the literature in which blockage of the lymph channels has been demonstrated to be due to lymphosarcoma, Hodgkin's disease and metastatic carcinoma involving the mesenteric lymph nodes in which the clinical symptoms of steatorrhea were present, but the anatomical pattern demonstrated in the lymph nodes in this condition was not present.

I am glad to hear of the experience of Dr. Robinson in regard to ligation of the thoracic duct. The thoracic duct was dissected out in the case reported by Whipple and the 2nd case of Blumgart's series and no obstruction was demonstrated. We have found no cases in the literature in which obstruction of the thoracic duct has produced the picture we have demonstrated in this case.

#### EXPERIMENTAL OBSERVATIONS ON THE PATHOGENESIS OF HYPERTENSION.\*

Harry Goldblatt, Cleveland, Ohio.

*Abstract.* A summary of investigations on experimental hypertension due to renal ischemia carried out by the author and collaborators as well as other

\* By invitation of the Council.

investigators. (See Harvey Lecture, 1937-1938, page 237, reprinted in *Bull. New York Acad. Med.*, 1938, 14, 523.)

By constricting the main renal arteries by means of a special clamp devised for the purpose, persistent hypertension was produced in dogs and monkeys which resembles human essential hypertension. The elevation of blood pressure is not immediate, but usually manifests itself in about 24 hours after the production of renal ischemia. Other investigators have now produced hypertension in rabbits and rats by the same method. Some of the dogs have had hypertension for more than 6 years. The type of hypertension which results depends on the degree of constriction of the renal arteries. When one main renal artery is constricted the blood pressure also becomes elevated, but the hypertension does not usually persist for more than a few weeks. In order to make the hypertension persist it is necessary also to constrict the other main renal artery or to remove the other kidney. In animals with hypertension due to constriction of one main renal artery the blood pressure falls to normal in 24 hours, or less, if the ischemic kidney is removed or if the clamp is released. When the renal ischemia is moderate there is no accompanying disturbance of renal excretory function, and the experimental hypertension resembles the benign phase of human essential hypertension. When the constriction of both main renal arteries is marked the excretory function of the kidneys is reduced and degenerative, necrotizing and inflammatory lesions of the arterioles develop in various organs. This resembles the malignant phase of human essential hypertension and also eclampsia.

Moderate constriction of the abdominal aorta, just above the site of origin of both main renal arteries, causes little or no immediate rise of blood pressure, but in about 24 hours hypertension develops without accompanying disturbance of renal excretory function. If the constriction is very great there is disturbance of renal excretory function, as well as hypertension, and pathological changes develop in the arterioles of many organs similar to those following great constriction of both main renal arteries. Constriction of the abdominal aorta just below the origin of both main renal arteries is not followed by elevation of blood pressure, either immediately or later. Thus, in dogs, the hypertension induced by the constriction of the abdominal aorta just above the main renal artery also appears to be of renal origin. Rytand, who worked on rats, came to the same conclusion about the origin of cardiac hypertrophy which developed after constriction of the abdominal aorta above the renal arteries.

The investigations that have dealt with the pathogenesis of experimental hypertension due to renal ischemia have eliminated a nervous reflex mechanism from the kidney as the cause of the hypertension. The present conclusion is that a humoral mechanism of renal origin is responsible for the increased peripheral resistance that determines the elevation of blood pressure. What the nature of the responsible substance is, how it is formed, and exactly how it acts are not yet known. The development of the hypothetical effective chemical substance is presumed to be due to the deficient irrigation of kidney tissue with blood. Whether some substance originally in the blood becomes altered, or accumulates in undue quantity, or whether something is imparted to the blood by the kidney tissue is not yet known. The part played by the organs of internal secretion known to produce pressor substances is not yet elucidated, but there is some indication that the integrity of a portion of adrenal cortex sufficient to sustain life is necessary if hypertension due to

renal ischemia is to persist. The same cannot be stated unequivocally for the hypophysis.

OBSERVATIONS ON THE EFFECTS OF RENAL ISCHEMIA IN PREGNANT DOGS AND RABBITS. C. C. Erickson and L. V. Dill (by invitation), Durham, N. C.

*Abstract.* Utilization of the Goldblatt clamp technic offered a method of studying the effects of renal ischemia in pregnant dogs. In addition to the expected hypertension and the renal lesions of ischemia, an increased susceptibility of the pregnant animals to renal ischemia was indicated, and lesions of the liver were demonstrated at autopsy.

A comparable syndrome — renal ischemia in pregnancy — has been studied in rabbits. Constriction of the renal artery was produced by a silver wire loop. Control animals included non-pregnant females, pseudopregnant females, ovariectomized rabbits, and pregnant and non-pregnant rabbits with complete arterial constriction. The significance of pregnancy associated with renal ischemia was emphasized by the greater susceptibility of the pregnant rabbits as indicated by survival time. Conspicuous lesions of the liver were demonstrated more frequently in the pregnant rabbits.

The observations suggest a correlation between the physiological and pathological processes induced by renal ischemia in pregnant animals with those in human eclampsia.

#### *Discussion*

(Dr. E. T. Bell, Minneapolis, Minn.) I think this is an important contribution to the study of eclampsia, and I think it can be compared to pregnancy in a person with a high degree of renal insufficiency. The most delicate functional test of the kidneys is pregnancy. If a woman has a latent chronic glomerulonephritis and becomes pregnant, the phenomena of eclampsia develop very readily. I think what Dr. Erickson has done is to produce a moderate renal insufficiency, which is entirely comparable with a low grade of chronic glomerulonephritis, and that this brings on the picture of eclampsia. The symptoms of eclampsia are almost identical with those of renal insufficiency.

(Dr. Harry Goldblatt, Cleveland, Ohio.) I purposely omitted any reference to the experiments on this subject which have been carried out by Dr. J. R. Kahn and myself, because I knew that this paper would follow my talk. We have allowed hypertensive animals to become pregnant and have found that the blood pressure has tended to go down, rather than up, during the pregnancy. After parturition the blood pressure has risen again to the previous hypertensive level, or even higher. Although it is true that in a pregnant dog one can produce all the lesions described by the authors, which are similar to the lesions of the "malignant phase" we have already described, yet it is equally true that we have observed the development of identical lesions in uremic and hypertensive non-pregnant females, and even in males, after excessive constriction of the main renal arteries. That the pregnant animal requires less constriction of the main renal arteries in order to produce these lesions, we can neither confirm nor deny on the basis of our experience up to the present time. As a possible explanation for the fall of pressure which we have observed in hypertensive dogs that have become pregnant, we have suggested that perhaps the fetuses with their normal



kidneys may be compensating in some way for the effect of the chemical substance which is responsible for the hypertension, as in the case of unilateral renal ischemia with the other kidney normal. Our own view at present is that there is nothing specific about the pregnancy that is responsible for the development of the eclamptic lesions in animals in which the malignant phase of experimental hypertension is produced by excessive constriction of the main renal arteries. In most other respects our results agree with those reported by Dr. Erickson and Dr. Dill.

(Dr. J. Loesch, Oneonta, N. Y.) About 14 years ago I produced persistent hypertension in dogs. Some of the animals became pregnant during the experiment. As marked nitrogen retention occurred, I fed these animals a low protein diet. Thus a load was taken away from the kidneys with the result that the animals did not die but survived. Otherwise they would have died from uremia. My work agrees with the observation of Dr. Erickson and Dr. Dill. The rest of my experiment showed about the same results as Dr. Goldblatt reported, whose paper I will discuss later.

(Dr. H. Edward MacMahon, Boston, Mass.) It would appear from the pictures Dr. Erickson has just shown us that he and Dr. Dill have successfully produced lesions in the vascular tree of the kidney resembling those seen in fatal chronic nephritic toxemia of pregnancy. The lesions within the liver are also of interest as they resemble those seen in eclampsia and rarely in uncomplicated malignant nephrosclerosis. Gross lesions of the liver in malignant nephrosclerosis are not common. A histological study of the liver in cases of malignant nephrosclerosis does show that this organ is quite commonly affected. Degeneration of isolated liver cells and active regeneration, as shown by the presence of numerous mitotic figures within the liver cells, are more commonly seen than the characteristic vascular lesions. Recently I had the opportunity to study sections from the liver of a middle aged male dying from malignant nephrosclerosis. Under low magnification little or nothing unusual could be seen within the liver cells. A more careful study demonstrated many mitotic figures in liver cells in all sections examined.

(Dr. Harry S. N. Greene, Princeton, N. J.) There is a spontaneous disease in the rabbit which bears a remarkable resemblance to eclampsia. In the rabbit, however, the blood pressure is lowered and the changes in the kidney are entirely degenerative in character. Such differences must be taken into consideration in attempts to induce eclampsia in this animal.

(Dr. Erickson, closing.) The only point I wish to comment on is Dr. Greene's mention of spontaneous eclampsia in rabbits. Certainly many of the lesions of the liver which he has described are quite similar to those we have seen in the pregnant rabbit with renal artery constriction. In this connection it is of interest that a similar spontaneous syndrome has been reported in one or two other animals, guinea pigs and sheep. In dogs, however, there is no similar spontaneous complex. Certainly the puerperal mastitis in dogs, which is sometimes described as an eclamptic complication, is not the same.

VASCULAR HYPERTENSION OCCURRING IN CASES OF COARCTATION OF THE AORTA. Hugo A. Freund, Detroit, Mich.

*Abstract not received.*

HYPERTENSION AND KIDNEY LESIONS PRODUCED BY X-RAY. F. W. Hartman, Detroit, Mich.

*Abstract.* As early as 1921 Romberg pointed out that where the maximum systolic blood pressure was constantly above 160 mm. Hg. kidney disease might be the cause. Fahr in 1925 expressed the view that hypertension was a compensatory mechanism and ran parallel with the amount of arteriolar damage in the kidney. In the same year Jaffé stated that there are many cases of hypertension with an isolated sclerosis of the small arteries of the kidney.

In communications on experimental nephritis produced by x-ray from 1925 to 1928, sclerosis of the smaller arterioles of the kidney, hypertension and cardiac hypertrophy were described.

Although an isolated kidney lesion as a cause of hypertension is now widely accepted, there is a definite need for a readily produced experimental renal arteriolar sclerosis in the study and solution of all the problems which this acceptance serves to emphasize.

Using smaller and repeated exposures of x-ray it has been found that the extensive fibrosis and replacement of all kidney structure as shown in previous work can be avoided. However, sclerosis of the intermediate and smaller arterioles, resulting in hypertension, cardiac hypertrophy, and finally kidney insufficiency, is produced.

HYPERTENSION FOLLOWING EXPERIMENTAL PERINEPHRITIS INDUCED BY CELLOPHANE. A PRELIMINARY REPORT. Irvine H. Page (by invitation), Indianapolis, Ind., and Irving Graef, New York City.

*Abstract.* It has been found that sterile cellophane, when gently applied, wrapped around the kidneys of dogs and secured either with paper clips or loose ligatures, produces an intense inflammatory reaction followed by a continuous fibroblastic and collagenous deposit. The inflammatory response produces a constrictive capsule 3 to 5 mm. thick around the kidneys. After several months the hilar structures, especially the renal vein, pelvis and ureters, may also be compressed.

From 2 to 3 weeks after the application the arterial pressure (measured by direct intra-arterial puncture) begins to rise and may reach a level of 240 mm. Hg. mean pressure after a month or two. In some animals the pressure reaches a peak and tends to fall to lower levels, while in others the pressure remained at high levels for 7 months (as long as the animals were being observed). Application of cellophane to one kidney caused hypertension, but not as marked as when both kidneys were treated.

At postmortem examination the kidneys, in short experiments, were found to be surrounded by a sanguinopurulent exudate in the form of a membrane between the true capsule and the cellophane. A similar exudate is present on the outer aspect of the cellophane. After 2 weeks the exudate around the cellophane continues to be sanguinopurulent and a dense fibroblastic and collagenous deposit appears on the true capsule. This increases to 3 to 5 mm. in thickness and extends around the entire kidney. At the end of 2 to 3 months the constrictive effect is marked and the kidney may be rotated in any plane and the hilar structures distorted and compressed.

The omentum becomes attached to the outer aspect of the cellophane and the exudate on the kidney. Dense adhesions which are richly vascularized and

the seat of marked histiocytic proliferation bind the kidney and the omentum together. Histological examination of the kidney reveals negligible changes in the first 2 months. Later, in the period of observation thus far used, marked compression with focal corticotubular atrophy and cortical scarring of the ischemic type become apparent. This is sometimes visible macroscopically, but is more marked on microscopic study. The glomeruli are remarkably well preserved in most instances. The collagenous hull which forms external to the normal capsule is readily separated from it and the true capsule shows little or no change. It in turn may be readily stripped from the underlying parenchyma.

The fate of cellophane is as yet undetermined. After long sojourn it may be found crumpled or broken into large pieces caught between dense fibrous deposits. When removed it appears to be unaltered and the inflammatory response persists around it. There is remarkable lymphoid hyperplasia in the nodes of the omentum; sometimes they exhibit acute lymphadenitis.

Hypertension produced by this method occurs whether the normal capsule is stripped or not before the application of cellophane. Denervation of the kidneys also does not interfere with its development. Removal of the "offending" kidney in animals in which hypertension has occurred after applying cellophane to one kidney causes the hypertension to disappear if it has not persisted for a long time.

Since hypertension may appear as early as 10 days after the application of cellophane to a kidney, it seems likely that the mechanism of its production is independent of gross compression of the renal artery.

#### EXPERIMENTAL ACUTE HYPERTENSION FROM OBSTRUCTION OF THE AORTA.

Robert Brotnier (by invitation) and E. T. Bell, Minneapolis, Minn.

*Abstract.* Goldblatt found that constriction of the aorta above the kidneys produces chronic hypertension, while constriction below the kidneys does not. The hypertension resulting from constriction of the aorta above the kidneys is attributed to renal ischemia.

This idea of renal ischemia as a cause of hypertension has been extended by some writers to include the hypertension in the upper extremities which results from coarctation of the aorta.

Using dogs under nembutal anesthesia the blood pressure was recorded on a kymograph with a cannula in the carotid artery. When the aorta was clamped above the origin of the celiac artery the blood pressure rose immediately, the average rise being 56 mm. Hg. On release of the clamp the blood pressure fell immediately to its previous level. When the aorta was clamped between the renal arteries the average rise of blood pressure was 13.5 mm. Hg., and when clamped below the kidneys, 8.8 mm. Hg. Clamping of both renal arteries did not affect the blood pressure.

It is concluded that the sudden rise of blood pressure following constriction of the aorta is due to a mechanical factor and not to renal ischemia. It is noteworthy that the degree of hypertension is directly related to the amount of blood obstructed. According to the work of Levy and Blalock, 60 per cent of the blood is obstructed by a clamp applied above the celiac artery and only 13 per cent by similar obstruction below the kidneys.

It is inferred that congenital stenosis of the aorta at the isthmus (coarctation) produces hypertension by mechanical obstruction of the circulation and not by renal ischemia.

VASCULAR MEASUREMENTS IN HYPERTENSION. H. E. MacMahon, Boston, Mass.

*Abstract.* The problem was divided into six parts: (1) to make an anthropometric study of the arterial tree of the kidney in the non-hypertensive state; (2) to make a similar study in conditions showing acute transient hypertension; (3) to make a similar study in several of the chronic hypertensive states; (4) to compare the vascular measurements in the hypertensive state with those in the non-hypertensive state; (5) to compare the vascular measurements in the arterial tree of the kidney in benign and malignant nephrosclerosis; and (6) to evaluate the significance of these anthropometric findings on the maintenance and course of prolonged and lasting hypertension.

This work was based on an anthropometric study of the arterial tree of the kidneys from 100 individuals ranging from 20 to 65 years of age. These were divided into three groups: (1) those with average blood pressure readings; (2) those with transient and acute hypertension including acute glomerulonephritis, acute hyperthyroidism and eclampsia; and (3) those with prolonged and lasting hypertension, including subacute glomerulonephritis, subchronic glomerulonephritis, chronic glomerulonephritis, benign nephrosclerosis and malignant nephrosclerosis.

In the preparation and selection of material, two or more blocks were cut from the kidneys in each case. The handling of the tissues was kept as constant as possible, and to facilitate measuring, differential stains on serial sections were frequently employed.

Measurements were made with a small micrometer eyepiece set into the ocular of the microscope. For simplicity, and since the study was primarily of a comparative nature, all measurements were merely read as "measuring units," rather than in microns. Approximately 40 transections of vessels were measured in each case. Five measurements were made from each transection, comprising: (*a*) the total width, (*b*) the diameter of the lumen, (*c*) the thickness of the wall, (*d*) the media, and (*e*) the intima. The total number of measurements was well over 20,000.

From these measurements a mean-average arterial tree for each case was graphically constructed, and on the basis of these measurements and graphs a comparative study of the several groups was made possible.

A brief summary of the outcome of this work is as follows:

Anthropometric studies on the arterial tree of the non-hypertensive and acute transient hypertensive groups were alike and may be represented by identical curves.

In the non-hypertensive group the artery shows a slow, gradual progressive diminution in size in its course through the kidney, maintaining an "angle of convergence" of approximately  $15^{\circ}$ . The lumen, the wall, the intima and media likewise diminish gradually and slowly. The diameter of the lumen is always greater than the thickness of the wall. The relative thickness of both wall and media is slightly greater in the arteriole than in other portions of the arterial tree. The intima reaches its relative and absolute peak in the larger vessels.

In the chronic hypertensive group the vessels are large throughout and show a rapid decline with an "angle of convergence" of  $35^{\circ}$ . The lumen of the arteries is large, uneven and precipitous. In the arterioles the lumen is narrow and stenotic and its diameter is very much less than the thickness of

the wall. The wall throughout is thick and heavy. The media in the larger vessels shows eccentric hypertrophy, whereas in the arterioles the media is insignificant, stretched and nearly lost. The intima is thick throughout and rather uneven. It attains its relative and near absolute peak in the arterioles and smaller arteries respectively.

A comparison of the vascular measurements in the chronic hypertensive and non-hypertensive groups brings out several striking differences.

In chronic hypertension the arterial tree is larger and pursues a comparatively precipitous course, with a larger lumen in the artery and a smaller stenotic lumen in the arteriole. The wall throughout is thicker and reaches a greatly exaggerated relative peak in the arterioles and smaller arteries. The media of the larger vessels is much greater, the media of the arterioles is much less. The intima is thicker, less uniform throughout, and reaches its relative greatness in the arterioles and smaller arteries, in striking contrast to the relationships in the non-hypertensive group.

A comparison of the vascular measurements in benign nephrosclerosis and malignant nephrosclerosis shows many striking similarities. The vessels are very large and precipitous. The lumens follow a rapidly converging course, being very large in the large vessels and very narrow in the arterioles. The walls are large and thick. The intima reaches its relative peak in the arterioles. The media of both groups is identical. The differences in the two groups (benign nephrosclerosis and malignant nephrosclerosis) were slight. The intima of the arteriole and smaller artery in malignant nephrosclerosis is 30 per cent thicker than that of benign nephrosclerosis, and the lumens of the same size vessels are somewhat smaller.

The significance of these anthropometric findings in the mechanism of hypertension and especially in the maintenance and course of prolonged and chronic hypertension is as follows:

Acute and transient hypertension may be found in cases in which the vascular tree is normal. That is to say, a disturbed anthropometric relationship in the arterial tree is not a prerequisite to hypertension.

With prolonged hypertension there is first an increase in size of the arterial tree which varies with the degree and duration of hypertension. This increase in size is the result of a progressive dilatation of the lumen accompanied by an eccentric hypertrophy of both intima and media.

In prolonged hypertension the "angle of convergence" is progressively increased, permitting a greater force to strike the smaller branches of the arterial tree.

The intima of the arteriole then thickens, leading to a progressive stenosis of its lumen. This increases the peripheral resistance and aggravates the conditions already existing. An increasingly vicious circle is gradually set in motion to maintain and to increase the existing hypertension.

#### CHANGES IN THE LARGER ARTERIES ASSOCIATED WITH EXPERIMENTAL HYPERTENSION AND AZOTEMIA. L. L. Waters (by invitation) and M. C. Winternitz, New Haven, Conn.

*Abstract.* The lesions of arterioles associated with hypertension and azotemia as produced and described by Goldblatt have their counterpart in the larger arteries and veins. Here the medial elastic fibers are spread apart and blood is found extravasated from vasa made conspicuous by necrosis of their walls

and the immediately surrounding tissues. These experimentally produced lesions are of particular interest in view of the occurrence of hemorrhage, necrosis and fibrosis of the media of the human aorta associated with renal hypertension and nitrogen retention.

### *Discussion*

(Dr. Theodore J. Curphey, Westbury, N. Y.) I should like to ask whether those scars in the media showed perivascular round cell infiltration.

(Dr. Howard T. Karsner, Cleveland, Ohio.) I am much gratified to hear what Dr. Winternitz says about these lesions in the media because in studying a large number of aortas I have been deeply impressed by the fact that such things do occur, and fairly frequently. Thus his experience coincides with mine. I may, I hope, be permitted to make reference to Dr. Curphey's question. Not infrequently these lesions are accompanied by what is loosely called "round cell infiltration," but we should be more specific in identification of the "round cells." Cellular infiltrations in the neighborhood of syphilitic lesions are somewhat different from those found in these lesions. In syphilis the cells are principally lymphocytes, and in these other lesions, although lymphocytes are present, large mononuclear cells predominate. Plasma cells may be found in both.

(Dr. Winternitz.) There is one thing: we see very little active syphilis, and after it has subsided there is a scar, and with due deference to my old teacher, Dr. MacCallum, I agree one cannot tell whether it is due to a spirochete or a mule kick, but when the lesions are active I think it is definitely true, as Dr. Karsner says, that the cytology in the vicinity of the lesion is very helpful.

### *Discussion of Papers on Hypertension*

(Dr. Howard T. Karsner, Cleveland, Ohio.) At the 1938 meeting of the Association of American Physicians I reported, on the basis of measurement of the thickness of the aortic media in nearly 500 cases, that the media of hypertensives is definitely thicker than that of non-hypertensives in comparable age groups. The increased thickness is associated with a separation of the elastic lamellae and a greater degree of intricacy of pattern than is found in the controls. Since advanced age is accompanied by much the same changes, it was suggested that perhaps hypertension produces a precocious senility in the aorta. Haythorn and others have determined the gradual increase in amounts of calcium in the aorta as age advances. Sections of the same aortas previously reported were treated by Kóssa's silver nitrate method and examined for calcium. If the increased thickness of the aortic media were due to precocious senility, it is to be expected that the aortas of hypertensives would show a greater degree of calcification than the controls. Roughly estimating the calcification as absent, moderate or marked, admittedly only a rough quantitative determination, it occurs in about the same degree throughout all the decades in both hypertensives and non-hypertensives. Thus it can be stated that the increased thickness of the aortic media in hypertension is not a manifestation of precocious senility. Evidently other factors determine the increased thickness of the aortic media in the human subject of hypertension.

(Dr. J. Loesch, Oneonta, N. Y.) I was very much interested in Dr. Goldblatt's paper because about 14 years ago I did similar experiments myself.

They were reported at the meeting of the Federation of Biology, Section of Experimental Pathology, in Rochester, N. Y., in 1926, and published in the February 18th and 25th, 1933, issues of the *Zentralblatt für innere Medizin*.

The approach of my experiment, however, was slightly different. In 1925 I did some work on extirpation and exclusion of the spleen, and during this experiment I found it of advantage to transplant the spleen under the skin. This gave me a clue to apply the same method to the kidneys. I transplanted both kidneys under the skin, or one kidney, and removed the other later if a "one kidney dog" was desired. To assure quick healing all pericapsular fat and that around the kidney pedicle was removed, probably also all nerves. Later I made an incision on both sides of the pedicle and clamped the renal artery, probably with the latter also frequently the veins, for various lengths of time every 3rd day, beginning at 5 minutes and gradually increasing to 30 minutes. By doing this a fibrotic ring was formed around the vessels, resulting in ischemia. The latter was, however, marked during clamping, which was intended to imitate spasms in human beings. The blood pressure in these experiments rose gradually within a month to 200 mm. Hg. or higher.

I followed these experiments for about a year and the blood pressure remained elevated even when for several months no clamping was done. The diastolic pressure rose, although somewhat later, to about 90 mm. Hg. In the one kidney animal the blood pressure reached about 180 mm. Hg. or slightly above. I thought these experiments gave an explanation for the etiology of hypertension, at least in some cases, although Kylin had denied any relation of the kidneys to hypertension. On the one hand, Romberg was a proponent of the renal theory. Volhard explained it on spasms, and Fahr on ischemia; thus I was able to prove by these experiments that the renal and ischemic theory was right. In my experiments the increase in blood pressure was caused by a renal and extrarenal, but renally determined, factor. If in one kidney dogs clamping was done 30 minutes every day over various lengths of time, they died from uremia. If, however, I clamped the two kidney dogs, which were fed the same diet, they lived much longer or did not die at all. The histological changes were about the same as Dr. Goldblatt reported; thus I found marked cellular proliferations of the glomerular capsule and loops, and further, fibrosis and hyaline degeneration of the glomeruli. The smaller arterioles, especially the afferent vessels, revealed marked thickening of the wall with nearly complete obliteration of the lumen. The tubules showed distention, flattening and desquamation. The heart was markedly hypertrophic and the brain edematous, but the rest of the organs showed hardly any changes. My conclusion at that time was that I was able to produce experimentally persistent hypertension.

(Dr. Paul Klemperer, New York City.) May I ask to what Dr. Goldblatt attributes the return of the blood pressure to normal after unilateral constriction of the renal artery. Is it due to the development of collateral circulation, so that if one would be able to avoid the formation of collateral circulation the blood pressure would be retained, or do you attribute it to the excretion of a hypothetical pressor substance by the normal kidney?

(Dr. E. T. Bell, Minneapolis, Minn.) I want to congratulate Dr. Goldblatt on his brilliant work. He has presented it to us very modestly. He does not claim that he has solved the problem of primary hypertension. I hope we won't rest on our oars and conclude that this whole question has been settled. The argument has been going on for 25 years as to which is first — the vascular

disease or the increase in blood pressure. There are certain arguments in favor of the idea that hypertension is primarily due to vascular spasm. One of these is that brought out by Dr. MacMahon, that there are clinical cases of primary hypertension similar in all respects to ordinary cases in which there are no appreciable changes in the vascular bed of the kidneys. There may of course be different kinds of hypertension. There are other arguments. Cases of long standing chronic glomerulonephritis often show an arteriosclerosis of high degree. Unless we think the two diseases are associated, we must conclude that the prolonged hypertension has caused the arteriosclerosis. There is the further consideration that the disease in the kidneys in hypertension begins in the large arteries and progresses toward the smaller vessels. We never see arteriolar disease without disease of the larger arteries preceding it. There is the further consideration that in the early stages of primary hypertension the patient during sleep has a fall in blood pressure, indicating that a vasomotor phenomenon is concerned in the disease.

(Dr. Goldblatt, closing.) Mr. Chairman, it is impossible to give you an adequate discussion of the interesting papers presented this morning in the 5 minutes that have been assigned for this purpose, but I shall do my best.

Dr. Fox whispered to me, "What do you think is the nature of the humoral mechanism which you consider responsible for the hypertension?" I do not know the answer, of course, but I can say that I am now a believer in the humoral mechanism and think that something happens to the blood that is going through a deficiently irrigated kidney which results in the presence in it of some chemical substance or property that can effect increased peripheral vascular resistance. Whether it means that some part of the kidney actually contributes a pressor substance to the blood, or that a pressor substance which is ordinarily eliminated accumulates in the blood, or that a substance in the blood that is not ordinarily pressor is changed to a pressor substance by flowing through the deficiently irrigated kidney, or that it is due to the neutralization, elimination, or destruction of a natural depressor substance, I do not know; but these are at least some of the possibilities. I can assure Dr. Fox that all of these possibilities are being investigated with great care by ourselves and many other workers.

I have already discussed the paper by Dr. Erickson and Dr. Dill, but I would like to add that we have found that the development of pregnancy in dogs with renal ischemia, in which the elevated blood pressure had returned to a lower level, did not cause the re-elevation of the blood pressure. In order to re-elevate the pressure in such dogs, it was found necessary to increase the renal ischemia. The dog is a quadruped with abdominal contents hanging down, so that in the usual positions assumed by dogs a mechanical compression of the aorta, or increased constriction of the arteries and veins, is not likely to result from the pressure of a pregnant uterus. In a human female, in the last stages of pregnancy, when the uterus is greatly enlarged, it is at least possible that acute renal ischemia resulting from pressure on the aorta and main renal vessels may be the cause of the hypertension and renal excretory insufficiency characteristic of this condition. The fact that eclampsia usually develops very late in pregnancy is certainly suggestive. We have even dared to suggest that pregnant women in the state of eclampsia might be placed on a contrivance which would permit the uterus to hang down and thus relieve the acute renal ischemia that might exist. Dr. Weiser of Detroit has already tried this procedure in a few cases with apparently good results.



In regard to Dr. Freund's contribution, in which he arrived at the conclusion that the hypertension which accompanies coarctation of the aorta, even in the thorax, may be due to renal ischemia, I can only say that all of our attempts to produce persistent hypertension in dogs by constriction of the aorta within the thorax have been without much success due to various complications. We hope now by a different method to be able to study this problem. However, I do believe that the hypertension which occurs in the upper part of the body of dogs with the aorta constricted just above the main renal arteries in the abdomen is of renal origin. Freund mentioned that in young individuals with coarctation of the aorta in the thorax they found little or no cardiac hypertrophy. This interests me, because the young are not apt to have significant coronary arteriosclerosis with resultant myocardial ischemia which probably plays a part in the greater cardiac hypertrophy observed in older people with persistent hypertension from any cause. We have found that in dogs the degree of cardiac hypertrophy as a result of persistent hypertension is also not great, and have felt that a possible explanation may be the absence of significant coronary arteriosclerosis in the dog.

Dr. Hartman's contribution is valuable. That intrarenal arteriolar and glomerular disease is produced by the irradiation is important. The only drawback of the method is that it is difficult to control the degree and extent of pathological changes in the kidney, and that, like our animals in the malignant phase, Dr. Hartman's dogs usually die of renal excretory insufficiency. This does not offer an opportunity for the study of the benign phase of experimental hypertension which, in my opinion, is the more important phase.

The contribution of Dr. Page and Dr. Graef is also of considerable interest. The hypertension which follows the application of an envelope of cellophane around the kidney is due in all probability to compression of the kidney substance by the thick perirenal scar which forms, as well as to compression of the renal vessels at the hilus, both of which may produce renal ischemia. There may even be interference with venous outflow from the kidney, which Dr. Bell and Dr. Pedersen showed years ago may be a cause of at least temporary hypertension. In order to reduce the accessory circulation to the kidney we have used dried sheep's cecum to form a membrane around the decapsulated kidney. This did not induce the formation of such a thick perirenal scar as is produced by the envelope of cellophane, and reduced the accessory circulation to the kidney, which was our only purpose.

In commenting on the contribution of Dr. Brotchner and Dr. Bell, I can only say that while it is true that I may not have committed myself very definitely in my talk in favor of the view that the hypertension which develops after constriction of the aorta just above the main renal arteries in the abdomen is due to renal ischemia, yet I believe this to be the case. My own interest in ischemia goes back to 1923, when I was a pupil of Professor Starling and worked under the direction of Dr. Anrep in the Department of Physiology at University College, London, England. My first studies dealt with ischemia of the legs of dogs and the reactive hyperemia which follows the return of the normal circulation to the ischemic limb. In studying this problem it became necessary to determine the effect of clamping the aorta at various levels right up to its origin from the heart. I found what had been known to physiologists for a long time, namely, that if one clamps the aorta at various levels, the immediate effect is a rise of the blood pressure above the site of the clamp and an abrupt fall of the blood pressure below it. The more

cephalad the site of the constriction of the aorta, the greater the immediate rise of blood pressure above the site of the clamp. This is in keeping with Dr. Bell's results. Had Dr. Brotchner and Dr. Bell clamped the aorta within the thorax, the immediate rise of blood pressure above the site of the constriction would have been even greater. Their longest period of observation lasted 40 minutes. In order to conclude that the hypertension above the site of the clamp as a result of the constriction of the aorta is entirely of mechanical origin, they should have studied the effect of the blood pressure for a much longer period. I believe they would have found that the initial immediate hypertensive effect would have disappeared in a short while, and that only 24 or more hours later would the blood pressure have again risen and remained elevated at least for a few weeks. I do believe that the immediate effect described by Dr. Brotchner and Dr. Bell is entirely of mechanical origin and due to the sudden interference with the onflow of blood through the aorta. The later rise of blood pressure which occurs after 24 or more hours, as in the case of the hypertension which follows clamping of the main renal arteries, is in my opinion due to renal ischemia. As a matter of fact, by very great constriction of the aorta, one may actually produce renal excretory insufficiency, as well as hypertension, and all the arteriolar lesions of the malignant phase that can be produced by constricting the main renal arteries.

At this stage I may state that those who do not accept the renal origin of the benign phase of essential hypertension are usually willing to accept it for the malignant phase. I know this to be true in the case of Professor Volhard. In my opinion this last concession is not necessary, for all that need be admitted is the renal origin of the renal excretory insufficiency. Yet I do believe that both phases can be of renal origin.

I can make no special comment on the study of Dr. MacMahon, not having made a similar study. His conclusion is different from that of Moritz and Oldt, on the basis of their study, with which I agree. We therefore obviously have diametrically opposite views, either of which may yet be proved true by experiment but cannot be settled by discussion.

The paper by Dr. Waters and Dr. Winternitz is an illustration of a very careful study in morbid morphology. Their findings are in keeping with the little that we can contribute to this subject on the basis of an entirely inadequate study up to the present time. Dr. Winternitz, with his great interest in the pathogenesis of arteriosclerosis, is the right man to make a study of the changes which occur in the large vessels of animals in the benign and malignant phases of hypertension due to renal ischemia. I look forward to some valuable contributions from his department on the causative relations between the hypertension, the renal excretory insufficiency, and the changes in the aorta and the large blood vessels.

In reply to Dr. Klemperer's question as to why the blood pressure returns eventually to normal when only one main renal artery is constricted, I can say that there are at least two possible answers. One possibility is that the natural accessory circulation to the ischemic kidney becomes sufficiently prominent to compensate for the ischemia. It has been known for many years that the dog has an abundant potential accessory renal circulation through the capsule and from other sources which may become prominent and effective under these conditions. The other possible explanation is that the normal kidney may compensate in some way by eliminating or neutralizing the effect of the hypothetical effective substance that is responsible for the increased

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peripheral resistance. Both factors may account for this phenomenon.

In the time that was allotted to me for my own talk, it was impossible for me to cover the entire ground and to refer to all the experiments that have been done to elucidate the pathogenesis of essential hypertension. However, I would like to take this opportunity to mention the work of Homer Smith and Goldring which is exceedingly interesting to me. Their study, by indirect methods, of renal blood flow in cases of human essential hypertension, indicates that there is always some reduction of blood flow through the kidney. This constitutes valuable support to the view that renal ischemia may, after all, be the initial cause of both the benign and the malignant phases of essential hypertension.

**EXPERIMENTAL INFECTIOUS PHLEBITIS AND ARTERITIS.** P. M. LeCompte (by invitation) and M. C. Winternitz, New Haven, Conn.

*Abstract.* Various organisms, when injected into the wall of the femoral vein in the goat, give rise to a reaction the extent and character of which are determined largely by the virulence of the organism and the duration of the experiment.

The lesions in the wall of the vein vary from acute phlebitis, with or without thrombosis, to fibrous intimal plaques. Particular emphasis should be placed on lesions of the neighboring artery; these consist of early intimal proliferation, exudation with fibrin precipitation in the vicinity of the internal elastic lamella, and later of fibrous intimal thickening.

A possible vascular pathway for such transfer of infection from vein to artery has been demonstrated by injection methods.

**BLOOD CHOLINESTERASE IN RHEUMATIC FEVER.** Mark P. Schultz and (by invitation) Edythe J. Rose, Washington, D. C.

*Abstract.* Whole blood and blood serum were obtained at approximately 10 day intervals from 24 patients with rheumatic fever and from 21 suffering from various other febrile diseases during the course of their illness, and these specimens were examined for cholinesterase by the method of Ammon and Voss. The usual range of values was established by examining 31 apparently healthy individuals. During the course of febrile diseases other than rheumatic fever the cholinesterase fell far below this level and invariably failed to return to it until the patients had completely recovered, as indicated, among other considerations, by the absence of fever and the return of the erythrocyte sedimentation rate to within normal limits. In 20 patients with rheumatic fever, on the other hand, the cholinesterase in some instances remained at the usual level or above, while in others it fell during the acute exudative stages of the disease, but remained at usual levels or above during frequently protracted periods of subacute activity associated with progressive carditis. In each of the 6 patients with chorea examined, the values without exception were also unusually high. In 4 only of the rheumatic fever patients observed the cholinesterase dropped and, as in the other febrile diseases studied, failed to attain usual levels until recovery was established. These patients were all young adults who suffered severe arthritis, but none of them developed more than discernible signs of carditis and in none did other than minimal cardiac damage result. Of the 20 rheumatic fever patients

in whom unusually high cholinesterase values were observed, on the other hand, 10 died and severe carditis with resultant extensive cardiac damage was the rule. In rheumatic fever patients a correlation was observed between variations in the P-R interval of the electrocardiogram and in the cholinesterase level. Increases in the former were associated with diminishing values for the latter, and vice versa.

MOTILITY AND CHEMOTAXIS OF LEUKOCYTES IN HEALTH AND DISEASE. Morton McCutcheon and (by invitation) O. Tod Mallery, Jr., Philadelphia, Pa.

*Abstract.* The outcome of an infection depends in part on the rapidity with which polymorphonuclear leukocytes are mobilized in infected tissues, and this mobilization is possible only as long as leukocytes react to the presence of bacteria by moving toward them. In order to find out whether this reaction is impaired in disease, experiments were made *in vitro* on leukocytes from a series of acutely ill persons suffering from such diseases as pneumonia, typhoid fever and congestive heart failure. For comparison, experiments were made also on leukocytes of the observer and on leukocytes of a series of patients not acutely ill.

A small clump of bacteria — pneumococcus, *Staphylococcus aureus*, or typhoid bacilli (differences in attraction could not be demonstrated) — was placed on a glass slide and a drop of blood, obtained by puncturing the finger, was superimposed and allowed to spread between the slide and the coverslip. The preparation was observed with the microscope at 37° C. A portion of the clump of bacteria was placed in the microscopic field and the path of each leukocyte was recorded on paper with the help of a drawing ocular.

Records made in this way were analyzed to find, first, how rapidly the cells moved, and second, how direct a path they followed toward the bacteria (the directional response of cells is known as chemotaxis). Leukocytes from acutely ill persons were found on the average to move 30 per cent less rapidly than those of the observer and those of patients not acutely ill. Also, cells of acutely ill individuals did not move quite as directly toward the bacteria as did those of the observer. It is concluded from these experiments that in the ill patients both rate of locomotion and chemotaxis were reduced — motility greatly, chemotaxis only slightly. The results indicate that leukocytes were damaged in the acutely ill patients, and this alteration might be expected to hinder the mobilization of leukocytes in infection.

### Discussion

(Dr. Theodore R. Waugh, Montreal, Canada.) I should like to ask Dr. McCutcheon if he considered the possibility that the collection of the red cells in the large aggregations which occur in acute conditions might possibly have had some effect on the path of the leukocytes. It is well recognized that in acute conditions there is a rapid sedimentation velocity of the blood and this is attributed to agglutination of the red cells in large aggregations.

(Dr. Theodore J. Curphey, Westbury, N. Y.) Have you tried the leukocytes of the patient in the plasma of the observer to see whether there might be something in the humoral side of the blood that might influence the changes in the leukocytes?

(Dr. Paul R. Cannon, Chicago, Ill.) Is it possible to make any similar measurements of immature leukocytes? I assume that the leukocytes were ma-

ture, but I wonder if immature leukocytes would be attracted at a slower rate, and whether or not you have made any observations of that sort.

(Dr. Howard T. Karsner, Cleveland, Ohio.) Will Dr. McCutcheon take the opportunity to harmonize these observations with the phagocytic activity of the leukocytes?

(Dr. McCutcheon, closing.) The first question was whether the path of the red blood cells might be obstructed by agglutinated red blood cells in ill patients. I can only say that agglutination was not observed—probably it was not especially looked for. It seems to me a possibility that the rate of locomotion would be reduced. I do not think it would be greatly reduced.

The second question concerned the leukocytes of the patient in the plasma of the observer. Unfortunately I have no data on that.

In regard to Dr. Cannon's question on the study of immature leukocytes, I think this could very readily be done in rabbits by injecting saline solution into the peritoneal cavity, as Ponder has shown that if this is done repeatedly at short intervals the blood contains many immature leukocytes. Then it would be possible to compare their chemotactic response with that of mature cells.

In regard to Dr. Karsner's question, we have made no comparison of these studies with phagocytosis. I think it would be very interesting to do so.

THE PATHOLOGY OF HUMAN BRUCELLIASIS.\* P. B. Parsons, Mary A. Poston, and Bowman Wise (by invitation), Durham, N. C.

**Abstract.** In November, 1937, a report of pathological investigation of 4 cases of human brucellosis was made in a preliminary form before the Section on Pathology of the Southern Medical Association. In the 1st case complete clinical investigations were followed by complete autopsy. The other 3 cases were studied clinically and by means of excised enlarged lymph nodes. The 1st case came under observation presenting a clinical picture with laboratory findings indicative of infection with *Brucella melitensis*. This diagnosis was confirmed by culture of the organism from excised lymph nodes. The histological picture of the lesion of the lymph nodes was that of non-specific necrosis. At autopsy the lesions were those of Hodgkin's disease grossly and histologically, but *Brucella melitensis* was cultured from numerous lesions in pure culture. The other 3 cases were studied clinically and by means of excised enlarged lymph nodes without culture. In these cases the clinical diagnosis on histological study was Hodgkin's disease. In view of the association between the histological and gross lesions of Hodgkin's disease and the organism *Brucella melitensis* in the case studied at autopsy, the 3 cases just mentioned were restudied clinically and other lymph nodes were removed. These nodes obtained at a second operation were studied histologically and bacteriologically. The histological picture was again that of Hodgkin's disease, but from the nodes a pure culture of *Brucella melitensis* was obtained.

This experience suggested naturally a possible relation between chronic brucellosis and Hodgkin's disease. The work described in this report deals with further studies designed to test the working hypothesis that these two diseases may be of one etiology. Seven cases of clinical Hodgkin's disease have now been studied by biopsy and by culture of excised lymph nodes. Clinical diagnosis of Hodgkin's disease has been histologically confirmed in all 7 cases and from all *Brucella melitensis* has been grown in pure culture.

\* This work has been supported in part by the James A. Greene Research Fund.

A series of 50 control cases has been studied by the same technic, but in none of these has the organism of brucelliasis been obtained. One of the original 3 biopsied cases had a third biopsy after a period of about 1 year. The organism was not obtained from the culture of the lymph node, but the histological picture was not changed essentially from that of the originally excised node.

The work here reported is a part of a series of studies of human brucelliasis now in progress. The present state of its development warrants the drawing of but one conclusion, that is, that chronic brucelliasis of the glandular type prevalent in the area in which this work is being done cannot be differentiated from Hodgkin's disease by either histological or clinical methods. Since the histological method has always been regarded as the criterion of diagnosis of Hodgkin's disease, this fact would seem to be significant with regard to the basic nature of these two disease entities.

### Discussion

(Dr. Herbert Fox, Philadelphia, Pa.) In view of the fact that millions of lymph glands have been cultured and similar studies made, I wonder what the method for isolation of *Brucella* has been to get 12 per cent positive in these 115 cases. I know I have tried, and others have tried, and millions of lymph nodes of the Hodgkin's type have been cultured. I am afraid I cannot go along with the thought that every large cell which has been called a Sternberg type is necessarily finally diagnostic of that condition we know as Hodgkin's disease, because Hodgkin's disease is not only a group of these cells, but it is a great deal more. I also wonder whether it is fair to take that picture of Sternberg's, which he himself told me might have been tuberculous disease, and compare it with the original ones which Hodgkin himself left.

(Dr. William H. Feldman, Rochester, Minn.) I am very much interested in this report and would like to know what were the types of *Brucella* found. Dr. Parsons called them *melitensis*, and I should like to know the criteria for differentiating the type of organism.

I would also like to ask if there was any evidence of spondylitis in patients from which the material was obtained.

(Dr. Charles T. Olcott, New York City.) Were there eosinophils in these lesions of Hodgkin's disease, and were there any fibroblasts?

(Dr. Howard T. Karsner, Cleveland, Ohio.) Without necessarily accepting the validity of Gordon's test, I think it would be of interest to know what results were obtained in this series of cases, if the Gordon test was applied.

(Dr. Fritz Levy, Elkins, West Va.) I would also like to know if eosinophilic cells were found, and what kind of mitoses was found in those large cells. In 1920 I reported in a preliminary note about cell division that characteristically abnormal mitoses were found in the cells which usually are called Sternberg's or Dorothy Reed's cells. They belong to a group of cells which have plurivalent nuclei after abnormal cell division. When at the end of a normal bipolar mitosis the nucleus is divided, but the cytoplasm is not, we get binucleated cells; sometimes the two nuclei fuse and we get large cells with large nuclei of the same type. I agree with the first discussion concerning the point that we are not allowed to make the diagnosis only on these large cells with one large or more normal sized nuclei, because it is only a certain deficiency in cell division. While we find this type of cell often in Hodg-



kin's disease, we find the same type of plurivalent cells in many other conditions when cell division is disturbed. At least we have to make sure that we are dealing with plurivalent cells of endothelial origin.

(Dr. Wiley D. Forbus, Durham, N. C.) I have been much interested in this finding, and there are certain things I should like to say, especially since I have carefully studied all of the material. Dr. Parsons has emphasized the Dorothy Reed type of cell, I think, unduly. He has not said enough of the other features of these tissues which are, in my opinion, identical with those of Hodgkin's disease. Being at first naturally quite sceptical, in order to assure myself of the genuineness of this histological similarity, I took all of the cases of Hodgkin's disease which we have seen at autopsy in the past 10 years and went over them very carefully, grossly and histologically. From a study of all of the records I was unable to make a differentiation between the pathological anatomical picture which Dr. Parsons has shown you, and that presented by the 8 or 9 cases of Hodgkin's disease, that is, those which we have called Hodgkin's disease. There is the possibility that we do not know what Hodgkin's disease is. We are fully aware of the fact that there are a great many different stimuli which are capable of provoking this particular reaction in lymph nodes. That *Brucella* may be one of those agents I think there is no question. Whether or not what Dr. Parsons has presented to you is Hodgkin's disease is something which, frankly, remains to be proved. We are not resting on our haunches in the matter since the importance of determining the true significance of the striking pathological similarity between brucelliasis as shown to you by Dr. Parsons, and Hodgkin's disease as we have all seen it, is obvious.

(Dr. Ralph D. Lillie, Washington, D. C.) I should like to ask a question as to whether there was the characteristic reticulum deposit among these pale cells we see in the usual type of Hodgkin's disease.

(Dr. Parsons, closing.) The first question was on the culture methods. Miss Poston has done most of the culture work and has worked with it for many years. This is the method which she uses: beef infusion blood agar adjusted to pH 7.4. We also use liver infusion agar with blood added at the time the culture is planted adjusted to a pH of 6.8. No culture is considered negative until it has been given a trial for at least 2 weeks, both aerobically and anaerobically.

The second question is one which several people asked. I am sorry I did not mention that in addition to the Dorothy Reed or Sternberg cells there were great numbers of eosinophils present and considerable fibrosis throughout the nodes. There was none of the original or usual architecture one would expect to find in lymph nodes. There were a great many large reticulum cells which we see in Hodgkin's disease. These nodes have been considered as Hodgkin's disease, not only by members of our department but by others outside of Duke University.

In regard to the question about the identification of the organism: The first culture was identified as *suis*, and three of the others as the bovine variety by both the agglutination and by the agglutinin-absorption method. They were sent to the National Laboratories for identification. The rest have been identified by us by simple agglutination.

We have had no spondylitis that I know of. We have had an incidence of some 70 per cent pruritus, which may be significant, since that is often a clinical finding in Hodgkin's disease.

Gordon's test has not been done. We have done animal inoculations but I can give you no results as yet.

I am sorry I am not prepared to answer the question about the mitoses in the cells. We have had numbers of cells which had very bizarre, piled-up nuclei, as though they had incompletely divided, but I can go no further than to make that statement.

(Dr. Carl V. Weller, Ann Arbor, Mich.) It might be a good plan to let some of these cases circulate through the Lymphatic Tumor Registry. I think they would be a very good addition to that series.

THE PATHOLOGY OF ENCEPHALITIS IN MAN CAUSED BY THE VIRUS OF THE EASTERN VARIETY OF EQUINE ENCEPHALOMYELITIS. Charles F. Branch and Sidney Farber, Boston, Mass.

*Abstract.* An outbreak of equine encephalitis in eastern Massachusetts in August and September, 1938, caused the death of over 90 per cent of 248 horses affected. Between August 15 and October 1, 1938, 26 human beings from 1 month to 60 years of age died of encephalitis. Seventy per cent of the patients were under 10 years of age. Studies by Webster and Wright, and Fothergill, Dingle, Farber and Connerley showed that the causative agent was the virus of the eastern variety of equine encephalitis. This report is based on portmortem examination of 17 patients who died in from 1 to 22 days after the onset.

Marked edema and congestion of the brain and cord, flattening of the convolutions, pressure cone formation, generalized congestion of the viscera and pulmonary edema were conspicuous gross findings. Microscopic examination revealed a severe diffuse meningoencephalitis, most marked in the basal ganglions and in the brain stem, and characterized by widespread nerve cell destruction and accumulations of inflammatory cells in perivascular spaces, the meninges, and in the numerous areas of necrosis. The cellular infiltration, largely neutrophilic in character in the early lesions, became predominantly large mononuclear and lymphocytic 6 to 20 days after the onset, a change which ran parallel to the cell types in the spinal fluid. Involvement of small vessels with neutrophilic infiltration and fibrin deposition throughout the walls was one of the most striking features of the disease. Demyelination was found only where entire areas were destroyed in the inflammatory process. The cord was involved severely in only 1 case. No bacteria could be demonstrated in association with the early lesions. No inclusion bodies were found. The pathological picture simulates most closely that of St. Louis encephalitis.

*Discussion*

(Dr. Edwin F. Hirsch, Chicago, Ill.) I think an epidemic very much like this occurred last fall near Minot, North Dakota, and the lesions in the human brain were very much like those described by Dr. Branch. The material was tested at St. Louis to see whether the virus in this epidemic was like that of the St. Louis epidemic, but there was no confirmation. I wonder whether Dr. Clawson has anything to offer in that connection.

(Dr. Benjamin Clawson, Minneapolis, Minn.) No.

(Dr. Branch, closing.) As far as we know, this is the first demonstration of the eastern strain of equine encephalitis in the human being, and these I believe were adequately proved by Webster and Fothergill.

## TRAUMATIC BRAIN STEM HEMORRHAGES. E. A. Linell, Toronto, Canada.

*Abstract.* Brain stem hemorrhages due to head injury can be divided into four groups in accordance with their anatomical situation. These situations are: (1) the subependymal tissues of the third and fourth ventricles; (2) the dorsal tissues of the upper midbrain; (3) the ventral tissues of the upper midbrain, involving the oculomotor nerve nuclei; and (4) the tissues of the lower midbrain and pons. In this series of 21 cases, 6 were subependymal, 8 were in the upper midbrain, and 7 were in the lower midbrain and pontine tissue.

In the first group the nerve cells in the hypothalamus and the floor of the fourth ventricle were involved in the hemorrhage. In 3 of these 6 cases there was a depressed fracture of the skull and in 1 of the remainder a gunshot wound of the frontal lobes was the cause of the hemorrhages.

The hemorrhages in the upper midbrain are mainly of interest from the damage which is necessarily caused to the oculomotor nerve nuclei, showing one of the mechanisms responsible for oculomotor nerve paralysis in cases of head injury. Some of these cases suggest bruising against the tentorium cerebelli as a possible mechanism for the midbrain hemorrhage.

The traumatic hemorrhages into the lower midbrain and pontine tissue are, as a rule, more massive than in the other groups. These massive hemorrhages, with the edema which accompanies them, are probably an important factor contributing to death. In the majority of cases in this group the patient died within a few hours after the injury.

## A STUDY OF BRONCHIECTASIS IN FIFTY LOBECTOMY CASES. W. L. Robinson, Toronto, Canada.

*Abstract.* This represents a further study of the pathology of bronchiectasis based on fresh surgical material from 50 lobectomies for this disease. The findings more or less confirm those of our first presentation on 16 cases as reported in the *British Journal of Surgery*, 1933, 21, No. 82.

In bronchiectasis the essential pathological process is a persistent infection of the bronchial wall leading to destruction of the musculoelastic elements and also, to a more or less degree, the cartilage plates. It is not an ulcerocavernous process as the other elements of the bronchial wall, cartilage plates, mucous glands and bronchial arteries are left intact. An occasional microscopic ulcer, however, may be found. The process might very well be compared to that of syphilis of the aorta. The inflammatory reaction in the subepithelial and muscular layers is constant in its presence and in the character of its wandering cell.

The problem of the etiology of this disease centers around the factors which make for the establishment and persistence of the infection. The infecting organisms are many and varied. The infection is apparently not specific in type, although the character of the inflammatory reaction suggests this to be so. The establishment of the infection is not as obscure as the reason for the persistence of the infection once established. Stagnation of the secretions in the lumen of the bronchus undoubtedly makes for persistence of the infection. This may be brought about mechanically by complete or partial occlusion of the proximal end of the tube by a tumor, foreign body, inflammatory mass, and so on. It may also be brought about

by any malfunctioning of the bronchial tubes such as paralysis of the ciliated epithelium whereby they are unable to clear the tubes properly of mucus and foreign material. Vascular sclerosis of the bronchial arteries may also play a part. Finally, in those cases where mechanical blocks can be eliminated it is suggested that there might be a physiological block occasioned by the presence of an area of metaplastic squamous epithelium at the proximal end of the tube. This would act as a barrier to the expulsion of mucus and debris by the ciliated epithelium in the lower part of the tube.

Ordinary respiratory movements of the bronchi are sufficient when the wall is diseased to produce the dilatations seen in these cases.

### *Discussion*

(Dr. Max Pinner, Ithaca, N. Y.) I am interested in this paper, particularly in the statement that ulceration of the bronchial epithelium was found relatively rarely. From the cases I have studied I was under the impression that ulceration is a rather prominent feature and that large portions of the bronchiectatic cavity are involved. The bronchi are seen ulcerated throughout the entire wall, including all its structures, elastica, muscles and cartilage. Inflammatory infiltrations around the nerves in the bronchial wall are frequently seen. With this deep ulceration, which in the later stages is frequently partly replaced by intensive fibrosis, there occurs such a profound destruction of the middle sized and small bronchi that this is probably one of the very important causes of perpetuating the infection of the bronchi by preventing proper drainage. If one follows the bronchi in longitudinal sections one finds very frequently stenotic and dilated portions; in other words, bronchiectasis is frequently to a large extent bronchostenosis, and peripheral to the stenosis are seen bronchiectatic cavities.

The other point to which must undoubtedly be attributed the perpetuation of the infection is the almost constant simultaneous existence of upper respiratory infection, providing continuous infection of the lower portions. One remaining feature which should not be overlooked in bronchiectasis is the fact that one does not find bronchiectasis in an otherwise normal lung. The pulmonary parenchyma is always severely damaged, depending on the stage of the disease, by suppurating or organizing processes.

(Dr. William S. Stanbury, Hamilton, Canada.) During the past year I have studied 15 pneumonectomy and lobectomy specimens of bronchiectasis. Although our series of cases was of a younger age group, our findings are essentially the same as Dr. Robinson's. I think at this time, when there is an increasing tendency, particularly in the clinical literature, to emphasize extra-bronchial factors in the etiology of bronchiectasis, Dr. Robinson's paper is very timely, demonstrating, as it does consistently, the essential lesion in the musculoelastic coat of the bronchus. In our series the majority of the cavities were lined by perfectly normal ciliated epithelium with well defined basement membrane; ulceration, when it occurred, was superficial, and where it occurred the underlying bronchial wall already had been destroyed extensively. I feel with Dr. Robinson that these lesions of the musculoelastic coat are fundamental to bronchiectasis, and ulceration when seen can be considered only an incidental finding. To the last speaker I would say we have seen equally good bronchiectatic lesions in the normal, collapsed, emphysematous and pneumonic lung. I do not feel an extrapulmonary process has any definite constant relation to the etiology of bronchiectasis.

(Dr. Robinson, closing.) I notice Dr. Pinner was talking about autopsy material. I found it rather difficult in going over a few autopsy specimens to be sure when one had ulceration, but I feel that in dealing with fresh material we are pretty safe. I am only reporting surgical specimens. We found very few ulcers and these were small and superficial. There is nothing in our series to indicate that the process is an ulcerocavernous one. I am convinced of that, but I cannot argue further as I have not gone over any extensive autopsy series. This is purely a group of surgical cases. As to the suggestion about the persistence of infection, I am ready to accept that. I feel, however, there is something local, some change in the local condition which makes for persistence of the infection in the bronchi, rather than a general inflammatory reaction in other parts of the respiratory tract.

I am glad to find that Dr. Stanbury corroborates our findings.

AGE AND SITE OF OPERATION IN RELATION TO POSTOPERATIVE PULMONARY EMBOLISM. J. S. McCartney, Jr., Minneapolis, Minn.

*Abstract.* It is commonly stated that operations on the lower part of the abdomen are often followed by pulmonary embolism. This is true because of the age at which such operations are done. If operations on various parts of the body were uniformly distributed throughout all decades, pulmonary embolism would not appear to be most common after lower abdominal operations. To prove this statement large numbers of operations from a variety of sources were compiled according to age and the part of the body operated upon. This compilation showed that the majority of operations on the head and neck and appendectomies are done before the age of 30 years, and this explains why such operations are only occasionally followed by embolism. Herniorrhaphy is not infrequently followed by embolism, about one-half of such operations being done after the age of 30 years, when practically all the instances of embolism appear. The majority of biliary and abdominal gynecological operations are done after the age of 30 years and so, not rarely, embolism takes place. Suprapubic bladder and prostate operations are done only very rarely before the age of 50 years and as a result pulmonary embolism is quite frequent after such operations. Operations on the extremities are carried out rather uniformly through all decades and embolism occurs in all decades, but particularly so in the older age periods.

From a study of the postmortem records of 3661 operative deaths it was found that although fatal pulmonary embolism may occur at any age, its frequency increases with age. The postmortem records were not from the operations used in determining the time of life at which operations are done. The conclusion was reached that it is not so much the part of the body on which operations are performed that is the cause of the embolism as it is the age of the patients at the time operations are carried out.

*Discussion*

(Dr. Edwin F. Hirsch, Chicago, Ill.) I should like to ask if the source of the emboli was traced in this series of reports, and whether the upper extremities were as common a site of origin as the lower extremities.

(Dr. Kornel Terplan, Buffalo, N. Y.) I should like to know whether in Dr. McCartney's material there was any evidence of fatal postoperative thrombo-embolism following operations on the brain.

(Dr. Carl V. Weller, Ann Arbor, Mich.) My own impression of this material is that we have been shown simply that the emboli we know about are those which occur in individuals who have impaired circulation. Probably emboli are equally common at all ages, but only those which occur in individuals who have an impaired circulation become known to the pathologist. If you had substituted "fatal embolism" for the word "embolism" in your conclusions I would have been in full accord with you.

(Dr. McCartney.) We traced as far as possible the source of each embolism. We have a few instances of fatal embolism following operations on the lower extremities and some on the upper extremities. As is commonly the case in our embolisms, the site is in the lower extremities or in the pelvis. In only about two-thirds of the instances were we able to find the source because we could not do a sufficiently thorough dissection most of the time to find it.

In answer to Dr. Terplan, we have a few instances of embolism following operations on the head. We do not get many deaths following surgical procedures on the head, or at least, we do not get many postmortems.

I should have stated I was going to discuss fatal embolisms. I left out the non-fatal embolisms intentionally. If we consider the non-fatal embolisms then the figures are practically trebled. If we took all infarctions or non-fatal embolisms, it comes back to the question as to what percentage of embolisms of the lung are fatal. There are statements of anywhere from 10 per cent up.

(Dr. Weller.) Do you believe that embolism can occur in the lungs without infarction in individuals who have a normal circulation?

(Dr. McCartney.) I am quite sure of it.

(Dr. Weller.) So that if you took the non-fatal emboli you still would not have an equal quota of them present in the earlier years?

(Dr. McCartney.) Yes.

(Dr. Harry C. Schmeisser, Memphis, Tenn.) How large must an obstructed artery be for a pulmonary embolism to be fatal?

(Dr. McCartney.) That is a question because it involves a number of things—the presence or absence of previous pulmonary disease and the presence or absence of pneumonia. The common statement in the literature is that in animals it requires somewhere around 60 to 65 per cent occlusion of the pulmonary artery before death can result, but as I see it in animals, most of the investigators were dealing with young animals and not with animals of an age comparable to the human being, and they have presumably a sound cardiovascular system. We get fatal emboli from occlusion of one lung. Virchow recognized that. He also recognized that we can get death, perhaps not really sudden, but death, after a time (minutes or hours or days) from a shower of small emboli, and with these showers of small emboli we occasionally have infarction, whereas with the massive embolisms which occlude the branches of the pulmonary artery we do not have infarction. There is no time for an infarct to develop and it does take a little time to form.

(Dr. Terplan.) Did I understand that Dr. McCartney found the source of the fatal thrombo-embolism in all cases?

(Dr. McCartney.) In two-thirds of them.

(Dr. Terplan.) We found it rather difficult to be certain as to the sources of the massive thrombo-emboli occluding the main stem of the pulmonary

artery. Even most careful dissection of the peripheral veins not infrequently failed in our experience to detect a thrombus. Nor could we find any endothelial damage in the larger veins of the lower extremities which might have pointed to the site of detachment of the massive thrombus. To me, the sudden fulminating fatal thrombo-embolism appears quite problematic. May I ask Dr. McCartney what his opinion is as to the theory of Havlicek? In our more limited experience we still find that sudden fatal surgical pulmonary embolism occurs almost exclusively following operations within or near the abdominal cavity.

(Dr. McCartney.) I have read Havlicek's paper. It sounds too simple. The thing that comes up in that connection is that one does not expect to get thrombosis in individuals who are jaundiced, because the jaundiced individual is supposed to bleed, instead of develop thromboses, and by and large that is true, but we not infrequently get fatal and non-fatal embolism in individuals who are jaundiced. It is rare, but it does occur. I think undue emphasis has been placed on the pelvic and femoral veins as a source for embolism. There is considerable information in the literature that the primary thrombus is low down in the extremity, in the ankle or foot, and can propagate into the femoral and iliac veins. We may milk out the large clots, if we cannot dissect them, but the small primary thrombi in the legs we do not get. We cannot carry out a sufficient dissection to get at them.

THE RELATION OF THE INSULIN HYPOGLYCEMIC REACTION TO SHOCK. Warren C. Corwin (by invitation), Philadelphia, Pa.

*Abstract.* It was proposed to ascertain the relation of the insulin hypoglycemic reaction to shock by determining the presence or absence of hemoconcentration during "insulin shock" and by noting changes present in the viscera after death from large doses of insulin. Determinations of the hemoglobin content and the number and volume of erythrocytes in the blood of dogs and rabbits indicated that hemoconcentration does not occur incident to hypoglycemic reactions resulting from injections of non-fatal or fatal doses of insulin. Visceral evidences of capillovenous congestion and increased capillary permeability were not seen in animals after death by insulin hypoglycemia. The mechanism of death resulting from large doses of insulin is not the same as that of shock. The term "insulin shock" is confusing and should be abandoned.

*Discussion*

(Dr. Joseph Tannenber, Albany, N. Y.) I agree entirely with Dr. Corwin that insulin shock is quite different from common shock or wound shock by its pathological manifestations. Therefore, it might perhaps be wise to agree on another name for it. In experimental insulin shock the animals die of lesions in the brain with large areas of cortical cells being bleached out, and ganglion cells in other parts of the brain, especially in the medulla, being similarly affected. The lesions of the central nervous system are so predominant that they obviously are the cause of death. When rabbits were seized by convulsions during insulin shock I have, however, also observed vascular reactions which might be of some significance. In such cases the ear arteries became so constricted that it was frequently impossible to obtain

blood from the ear veins during or some time following a seizure, even when local irritants such as xylol were employed.

(Dr. Corwin.) I agree with Dr. Tannenberg. I should have emphasized that the term "insulin shock" is after all confusing and therefore should no longer be employed. Some such term as "insulin reaction" would be preferable. I also agree that the mechanism of death from large doses of insulin is primarily a cerebral one. It is undoubtedly due to the inability of the brain to utilize oxygen in the presence of a reduced glucose content of the blood. Recently studies on the protein content and osmotic pressure of the serum in man and the dog during insulin hypoglycemia have been published by Butt and Keys. These authors likewise concluded that so-called insulin shock bears no close relation to other types of shock.

STUDIES ON METAPLASTIC OSSIFICATION. Sheldon A. Jacobson, Brooklyn, N. Y.

*Abstract.* It has been demonstrated by Huggins and others that the transplantation of urinary epithelium will provoke metaplastic bone formation in the rectus muscle of the dog. By experiments on the rat it was found to be refractory to this procedure. In an attempt to change the animal's reaction, 170 experiments on 102 animals were performed as follows:

Bladder segments, dome of the bladder and minced bladder were transplanted into the muscle peritoneal sac, spleen, liver and kidney. Bladder transplants with: (a) subcutaneous injections of calcium chloride in the graft area; (b) calcium lactate 0.5 per cent as drinking water; (c) a 50 x normal therapeutic dose of viosterol; (d) a 500 x normal therapeutic dose of viosterol; (e) 0.025 mg. elementary phosphorus daily; (f) 0.05 mg. elementary phosphorus daily; (g) the same after destruction of one kidney by radium emanation seed; (h) implantation in the same area of boiled beef bone, boiled rat bone and 50 x normal viosterol and silver nitrate crystals were all tried.

Kidney segment, kidney cortex, kidney medulla, kidney pelvis and ureter transplants were made in muscle.

Boiled rat bone, boiled beef bone and silver nitrate crystals were also implanted.

The vessels of one kidney were ligated.

Removal, eversion and replacement of the dome of the bladder were performed.

Removal of the dome of the bladder and replacement by fascia were also done. Removal, eversion and replacement of the dome of the bladder and administration of elementary phosphorus were done. Bladder transplants were made in pregnant females.

These attempts were almost uniformly unsuccessful, there having been a very feeble bone formation in 5 scattered experiments.

The guinea pig was studied and was found to produce bone almost uniformly on transplantation of bladder tissue to muscle. In an attempt to ascertain whether the calcification was primary and acted as a stimulant to ossification, or whether the deposition of bone matrix was the first step in the process, an attempt was made to change the reaction of the guinea pig in this respect by the administration of a scorbutogenic diet. In no case was bone deposited nor was calcification observed.

Despite the refractoriness of this animal to rickets, the experiment was re-



peated with the animals fed a rachitogenic diet. Two out of 10 animals showed bone formation.

On the basis of histological pictures found incidentally in these experiments the theory of the histogenesis of osteochondroma and of the epiphyseal plate is suggested.

### *Discussion*

(Dr. Edwin F. Hirsch, Chicago, Ill.) I should like to ask if any appreciable amount of cartilage was found. I understand in the experiments which Huggins performed that there was no appreciable amount of cartilage associated with bone formation.

(Dr. Jacobson.) In the case of the dogs there was a large amount of cartilage in more than 1 animal. The slide I showed was not an incidental finding. I have a couple more slides but there was no point in showing them as they add nothing. I did not find cartilage in the guinea pigs.

**THE CAUSAL SIGNIFICANCE OF TRAUMATIC OSSIFICATION OF THE FIBROCAR-  
TILAGE IN TENDON INSERTIONS.** Edwin F. Hirsch and (by invitation)  
Russell H. Morgan, Chicago, Ill.

*Abstract.* The early stages of the lesion (11 cases) of traumatic ossification contain large amounts of fibrocartilage or hyaline cartilage continuous with bone in varying degrees of differentiation. Some portions seem to be ossifying cartilage, others have lamellar trabeculae containing residues of cartilage. The late stages of the lesion have a high content of lamellar bone and only small traces of cartilage. These conditions imply the origin of bone in a cartilage matrix that is endochondral bone formation.

Fibrocartilage is a normal constituent of the insertions of many tendons (69 from postmortem material) in which traumatic ossification occurs. A reactive or reparative growth of these tissues initiated by trauma provides a simple explanation for the lesion of traumatic ossification.

**POLYVINYL ALCOHOL STORAGE DISEASE.** W. C. Hueper, New York City.

*Abstract.* Polyvinyl alcohol is one of several plastic substances developed during recent years by the chemical industry, and is used in the manufacture of resins, lacquers, and so on. Polyvinyl alcohol, introduced as an aqueous colloidal solution subcutaneously or intravenously into rats and rabbits, respectively, is retained in the organism to an appreciable extent and for a considerable time at the site of injection, as well as in numerous remote organs. The injected chemical is stored (a) in the reticuloendothelial cells of the spleen, liver, suprarenals and lymph nodes; (b) in the endothelial cells of the blood vessels of the brain, lung and kidney (glomeruli); and (c) in histiocytes of various organs (lungs, chorioid plexus, testes, retroperitoneal tissue), in fat cells, and in ganglion cells and glia cells of the brain. The inner wall of blood vessels often is covered by a coat of polyvinyl alcohol which may result, especially in the lung, in the production of endothelial swelling and subsequent cellular damage characterized by the presence of foreign body giant cells and phagocytic foam cells containing polyvinyl alcohol. The polyvinyl alcohol present in tissues and cells can be demonstrated readily in

sections by its characteristic and specific blue color reaction with Lugol's solution. The character and extent of the organic lesions produced are related to the physicochemical properties peculiar to polyvinyl alcohol (large molecular size, viscosity of the aqueous solution, precipitability from a liquid, colloidal state to a particulate, solid state by changes of the salt concentration of the medium, tendency to form films, and marked resistance to chemical as well as enzymatic, metabolic degradation). Studies with polyvinyl alcohol may provide a method by which additional information may be obtained possibly in regard to the physicochemical action-mechanism of certain biologically important, macromolecular substances or aggregates (proteins, polysaccharides, lipids) under normal and pathological conditions (immunity reactions, malignant tumors, storage diseases, degenerative lipoidoses).

### *Discussion*

(Dr. Herbert S. Reichle, Cleveland, Ohio.) I thought that hitherto the endothelial cells of the larger vessels have not been regarded as a part of the reticuloendothelial system in the sense that they would be phagocytic. I noticed that in the vein of the lung the endothelium contained polyvinyl alcohol. Are you sure in this case there were not thrombi, either mural or agglutinative, and that the cells in these contained the polyvinyl alcohol?

There is a further point about the brain — the remarkable thing that the ganglion cells should contain this material — and the question arises whether this is a lipoidhistiocytosis in the sense of the words hitherto used, or whether this material is taken up by cells in general due to some metabolic change which we cannot define more closely.

(Dr. Carl V. Weller, Ann Arbor, Mich.) It seems to me the occurrence of this material in ganglion cells and glial cells, as well as in the histiocytes, is one of the most important things about this presentation because it brings to mind Tay-Sachs disease in which this distribution occurs.

(Dr. Hueper, closing.) I intended to take up the subject of phagocytosis in my paper, but on account of the lack of time I was prevented from doing so. I may therefore read the passages referring to this subject contained in my paper:

"The retained portion of the polyvinyl alcohol is stored in reticuloendothelial cells, endothelial cells, histiocytes, glia cells, and various other cellular elements which are ordinarily not included among the cells possessing phagocytic qualities. These are fat cells, fibroblasts, endothelial cells of larger vessels, ganglion cells, and so on. On the other hand, leukocytes, which are credited usually with pronounced phagocytic qualities, do not ingest this chemical in spite of an intimate contact with it. Mononuclear cells, however, may form an exception. Phagocytosis of liquid foreign matter does not seem to follow the same rule as to cellular types participating in this phenomenon as that established for the phagocytosis of solid particulate matter. The physicochemical properties of the particular substance involved determine apparently the types of cells which may take a part in phagocytic activities."

There can be no doubt that endothelial cells of larger vessels phagocytize polyvinyl alcohol, as this substance can be demonstrated in these cells by the specific reaction with Lugol's solution.

MUSCULAR DYSTROPHY IN BILIARY FISTULA DOGS. E. D. Warner and K. M. Brinkhous (by invitation), Iowa City, Ia.

*Abstract.* Progressive degeneration of skeletal muscles resulting in extreme paresis occurred frequently in a group of dogs with biliary fistulas of the gall bladder-renal type. The distribution and type of lesions of the muscle observed are comparable to those of the "nutritional muscular dystrophy" in rabbits and guinea pigs described by Goettsch and Pappenheimer. One sees Zenker's degeneration with connective tissue replacement of the necrotic muscle cells and marked atrophy of fibers which do not undergo necrosis. No lesions have been found in the nervous system. The disorder is thought to result from a dietary deficiency incident to the absence of bile in the intestine. The animals were fed a mixed diet which has been entirely adequate for animals not having biliary fistulas. Simple inanition can be excluded as the cause of this condition.

ACUTE HYPERTROPHIC HEPATITIS. J. D. Kirshbaum and (by invitation) H. P. Popper, Chicago, Ill.

*Abstract.* Fifteen cases of acute primary parenchymatous jaundice are described. Clinically they were characterized by a fulminating fatal course, death usually occurring within 10 days, and only 2 cases showed a subacute course of 1 to 3 months duration, but without any signs of healing. The onset with severe jaundice was frequently accompanied by fever, chills, gastro-intestinal signs, pains in the muscles and joints, and leukocytosis. Later there were cerebral manifestations associated with nitrogen retention and anuria. In the etiology drug poisoning was excluded, but there were mentioned in the history food poisoning, upper respiratory infections, gonorrhea, and in 1 case *Paratyphoid B* was isolated. Clinically a diagnosis of hepatitis was suggested in some cases. Despite the similarity of the clinical picture to acute yellow atrophy of the liver, it was differentiated by the marked enlargement of the liver, as seen in catarrhal jaundice. At autopsy the large liver (average weight 2150 gm.) was striking. The spleen was enlarged with increased fibrosis, congestion and focal aggregations of erythrocytes simulating hemorrhages. The kidneys were swollen and showed microscopically degenerative signs. Histologically the liver showed: first, damage to the cells with necrosis and necrobiosis, indicated by disappearance of liver cells, presence of granular debris, bile storage in the cells, collapse of the framework, and also fatty changes. The changes were mostly localized in the center of the lobules. Second, a marked toxic edema was visible with enlargement of the spaces between the blood capillaries and the liver cell cords in which coagulated plasma proteins could be demonstrated. Various degrees of such a serous hepatitis (Roessle, Eppinger, Popper) due to damage of the blood capillaries of the organ produced a disturbance of the structure of the cell cords with segregation of single or groups of cells—so-called dissociation. That the dissociation is not a primary parenchyma cell process is revealed by the intact nuclear staining of the round shaped dissociated cells. Sometimes the parenchyma cells are entirely washed away and appear in the lumen of the veins. Attempts at healing with regeneration of liver cells and proliferation of bile ducts were seen in Case 16. Jaundice appeared subsequent to an alimentary intoxication which, however, decreased. Death was due to an

ulcerative colitis. Thus, we are dealing with a transitional stage between catarrhal jaundice and acute atrophy of the liver with damage to the parenchymatous cells and the capillaries. The latter in the form of a serous hepatitis causes the enlargement of the organ, and the same explanation may be valid for the enlargement of the liver in catarrhal jaundice. The cause of the icterus is a combination of a localized breaking up of the liver cell cords due to necrosis or dissociation with a consequent communication between bile capillaries and tissue spaces, and a generalized functional damage is produced by the serous hepatitis. The latter prevents the excretion of the regurgitated bile occurring in necrosis alone. This explains all forms of parenchymatous jaundice. The presence of the toxic edema would indicate active therapy for dehydration of the liver by means of intravenous injections of hypertonic sugar solutions in all types of parenchymatous hepatitis.

### *Discussion*

(Dr. Carl V. Weller, Ann Arbor, Mich.) This is a type of case that bothers us from time to time, particularly from the standpoint of etiology. I always suspected there were unknown toxic factors rather prominent in this group. Often, particularly in children, we have been unable to determine what the toxic factor might be, in spite of efforts at detective work.

(Dr. Kirshbaum.) The child presented showed *B. typhosus* bacteriologically. That was the only case in which we were able to identify organisms.

### THE DEVELOPMENT OF HEPATIC CIRRHOSIS FOLLOWING HYPOPHYSECTOMY.

Irvine H. Page (by invitation), Indianapolis, Ind., Irving Graef, New York City, and (by invitation) Joshua E. Sweet, New York City.

*Abstract.* During observations of the effect of complete hypophysectomy on the induction of hypertension by renal ischemia (after Goldblatt), 2 dogs (male and female) were kept alive  $2\frac{1}{2}$  years. They developed diabetes insipidus, marked adiposity, loss of gonadal function, and changes in their coats. At postmortem examination nodular hepatic cirrhosis was encountered and cortical atrophy of the adrenal glands, as well as involutional changes in the gonads.

Three additional hypophysectomized dogs (furnished by Dr. Bodo), with a similar clinical syndrome and surviving 9 months to 1 year, exhibited milder grades of portal cirrhosis. Fatty change was present in all but was not remarkable.

The endocrine relation of the liver to the pancreas is well known, but a relation to the pituitary gland and its nervous connections is not well established. In human pathology, Wilson's disease, the occurrence of gynecomastia, and loss of hair in cirrhosis of the liver, are findings which imply a relation that may be reciprocal.

### VITAMIN A STORAGE IN ACTIVE AND ARRESTED CIRRHOSIS. Alvin J. Cox, San Francisco, Cal.

*Abstract.* In 43 fatal cases of cirrhosis of the liver the vitamin A content of the liver, as measured by the antimony trichloride reaction, was studied in relation to the clinical course of the disease and the character of the lesions in the liver. The livers in which the pathological process showed the greatest

activity contained practically no vitamin A, whereas when the lesions showed little or no evidence of activity the stored vitamin A approached the average normal value.

### *Discussion*

(Dr. James S. McCartney, Minneapolis, Minn.) I should like to know how many of these cases were patients who died of cirrhosis — whether any of these were the so-called accidental findings at postmortem, and whether Dr. Cox found, if they fell into those two groups, degenerative changes, or, as he spoke of it, signs of activity in the livers of individuals which have cirrhosis as an accidental finding.

(Dr. Carl V. Weller, Ann Arbor, Mich.) I wonder whether the use of the term "precirrhotic" for the degenerative changes here would not have made the presentation a little clearer. I think of cirrhosis as beginning with fibrosis, so that it seems to me that if you had said precirrhotic and cirrhotic activity I would have understood it a little better.

(Dr. Cox.) In answer to Dr. McCartney's question, I am sorry I have not prepared a table correlating symptoms of cirrhosis and vitamin A content of the livers. In some of the cases cirrhosis was not a cause of death. There was good correlation between the clinical and the anatomical pictures. Most of those cases with death from esophageal hemorrhage or intercurrent disease, or without the clinician having been aware of the cirrhosis, fell into the group of arrested cirrhosis and showed little evidence of degeneration of liver cells.

EXPERIMENTAL TROPICAL CIRRHOSIS. Philipp Rezek (by invitation), Miami, Florida.

*Abstract.* Experimental studies on tropical cirrhosis are reported. The peculiar so-called Indian infantile cirrhosis is described. Different forms of hepatitis were produced by feeding dogs with the hot spices that constitute part of the daily diet in British India.

Alterations in the livers of newborn puppies from females who had been fed with these Indian hot spices during pregnancy and lactation were also found. These alterations are similar to those found in newborn infants in India suffering and dying at the rate of 15,000 to 20,000 annually from this type of cirrhosis. It is believed that there is a field for tropical research in the study of hot spices in relation to the intra-uterine effects on the newborn and effects during lactation.

It is also believed that further chemical analyses of condiments would be of help, since epilepsy of the human type in dogs, after administration of pyrol, a substance contained in pepper, was produced.

### READ BY TITLE

REPORT OF A FATAL CASE OF HISTOPLASMOSIS IN INFANCY. Arthur L. Amolsch, Detroit, Mich.

THE SPECIFICITY OF THE HUMAN INFLUENZA VIRUS FOR PULMONARY TISSUE, AS SHOWN BY EXPERIMENTS ON MAMMALIAL FETUSES. N. Paul Hudson and (by invitation) Oram C. Woolpert and Herman A. Dettwiler, Columbus, Ohio.

EXPERIMENTAL LESIONS OF ARTERIES WITH HUMAN FAT, FATTY ACIDS, SOAPS AND CHOLESTEROL. Oscar O. Christianson (by invitation), Tuscaloosa, Ala.

THE ETIOLOGY OF CONGENITAL BILATERAL POLYCYSTIC KIDNEYS. James E. Davis, Ann Arbor, Mich.

REFRACTORY ANEMIA PRODUCED BY DIFFUSE PLASMOCYTOMAS. Walter W. Jetter, Buffalo, N. Y.

THE INFLUENCE OF CUTANEOUS INFLAMMATION ON THE ACTION OF STAPHYLOCOCCUS TOXIN. Harold B. Kenton, Chicago, Ill.

CYSTS OF THE THIRD VENTRICLE. E. A. Linell, Toronto, Canada.

RUPTURED CONGENITAL ANEURYSM AS A CAUSE OF INTRACEREBRAL HEMORRHAGE. E. A. Linell, Toronto, Canada.

LESIONS OF THE MYOCARDIUM ASSOCIATED WITH A LOW POSTASSIUM DIET. Ernst Mylon (by invitation), R. M. Thomas and M. C. Winternitz, New Haven, Conn.

*Abstract.* White rats, fed a diet which limited the potassium intake to 1.2 to 1.5 mg. per day, lost weight steadily and lived on this diet for an average of 29 days. Control rats on the same basal diet with added potassium chloride (12 mg. per day) increased in weight during the period of the experiment and seemed healthy in every respect.

The only lesions of significance found in the animals fed low potassium diets were confined to the myocardium. This showed focal and diffuse areas of necrosis with replacement of the muscle fibers by young cellular connective tissue. The lesions, when extensive, were chiefly subendocardial and not infrequently complicated by mural thrombi.

ACTIVE ENDOCARDITIS IN THE NEWBORN (REPORT OF 2 CASES). Alfred Plaut, New York City.

*Abstract.* In a previous paper (*Arch. Path.*, 1935, 20, 582) we were able to record what we considered the first microscopic evidence of active endocarditis in the newborn. A second instance of this probably extremely rare condition has come to our attention.

On the tricuspid valve of a 1 day old male baby, who died of a subarachnoid hemorrhage, generalized hyperemia of the brain, suprarenal hemorrhage and atelectasis of the lungs, was a small, brittle, grayish cluster, 5 by 2 mm., attached to the edge of the anterior leaflet and surrounding the contiguous chordae tendineae. There were no other unusual lesions in the heart. Two pin-point blood cysts (so-called valvular hematomas) were seen in the mitral valve.

A search was made for foci of infection in the organs. A few small vessels in the kidneys were found distended with fibrin-like masses and a few leukocytes. Numerous small vessels in the lungs, some of them definitely of arterial character, contained masses similar to those in the kidney. Occasionally there was a slight overgrowth of the endothelium of these vessels. In wide thin walled veins at the lateral edge of the thyroid gland older thrombotic masses

were found consisting of rather compact fibrinoid material and numerous, partly mononuclear partly polymorphonuclear cells. At two points the thrombotic mass was found attached to the vessel wall. A thick walled vessel in a villus of the placenta also contained a fibrinous mass with a few leukocytes. The hemorrhages which were found in different organs, including the sub-epicardial tissue, consisted almost exclusively of red cells, in marked contrast to the intravascular masses containing numerous leukocytes.

The microscopic picture of the endocarditic lesion was somewhat different from that in our 1st case. The endocarditic lesion consisted mainly of fibrin which at several points was adherent to the valve, the superficial layers of which were ulcerated at these points. Leukocytes were scarce in the fibrinous mass, but the loose hemorrhagic masses surrounding it contained many. The nuclei in the valve near the endocarditic lesion were numerous and swollen. No leukocytes were seen in the valve itself. No microorganisms were found in crush smears of the vegetations and in the paraffin sections. Aerobic and anaerobic cultures of the spleen remained sterile.

As in our previous case, the pathogenesis of the endocarditis remained obscure. But while in the 1st case no other inflammatory lesion could be found, there was in the 2nd case evidence of inflammation. The thrombus in the neck vein obviously was not recent. The thrombotic masses in the lungs and kidneys appeared to be more recent, but the endothelial overgrowth in vessels of the lung also denoted a certain duration. Unfortunately there is no record of the condition of the middle ears.

No infectious disease of the mother during pregnancy was noted. She had high blood pressure and edema of the feet. The membranes ruptured 24 hours before onset of labor.

The fact that the mother had no symptoms of infectious disease during pregnancy is not astonishing, since the mother may remain in good health even in true sepsis of the fetus (Wohlwill and Bock, *Arch. f. Gynäk.*, 1928, 135, 271, Case 3). The possibility of an infectious focus in the neck or the head should be considered on account of the adherent thrombus in the neck vein. The most common inflammatory process in a newborn we might think of in this connection would be otitis media. In the cases reported by Hemsath (*Arch. Otolaryng.*, 1936, 23, 78), however, no mention is made of evidence of inflammation in other parts of the body with the exception of so-called pneumonia of the newborn. We have examined the organs of 36 newborns in an attempt to find similar intravascular thrombotic and leukocytic masses but with negative results.

These 2 cases of active endocarditis in an otherwise normal heart, on normally formed valves, represent something different from the postulated endocarditic processes whose sequelae are found on malformed valves. What would become of such an endocarditis in case the infant survived remains doubtful. The case of Püschel (*Arch. f. Kinderh.*, 1938, 114, 1) seems to indicate that such an endocarditis might heal without major defects in the valve.

A RARE CASE OF NEUROFIBROMA OF THE MESENTERY RESECTED DURING THE LATTER MONTHS OF PREGNANCY FOLLOWED BY UNEVENTFUL RECOVERY AND NORMAL DELIVERY. S. H. Polayes and (by invitation) J. A. Timm, Brooklyn, N. Y.

*Abstract.* A colored female, aged 20 years, para I, gravida II, and 6 months pregnant, was admitted to the Cumberland Hospital on Oct. 24, 1938. For about 1 month prior to admission she had been conscious of an enlarging epigastric mass above the womb. This was accompanied by attacks of abdominal pain, backache, vaginal discharge and bleeding.

Neither the past nor the family history was remarkable. Physical examination revealed small nodules deep in the skin of the neck and abdominal wall, and a non-tender, freely movable, large, firm, ovoid mass situated in the mid-upper abdominal region. A fetus of about 6 months gestation was visualized in utero by x-ray examination. When a miscarriage appeared imminent a laparotomy was performed and a large segment of ileum and a tumor of its mesentery were removed. A side-to-side anastomosis of the remaining ileum was then performed. The patient made an uneventful recovery and later was delivered of a full term normal child.

A summary of the pathological report of the tissue removed at operation is as follows:

The specimen is a coil of ileum, 60 cm. long; the wall and mucosa are thickened; the mesentery is 8 cm. thick and composed of discrete, pearl gray, glistening firm nodules, some of which are cystic and discolored red and blue by hemorrhage. Microscopic examination of the specimen reveals numerous groups of ectopic sympathetic neurons lying immediately beneath the lining mucosal epithelium, the latter being in areas composed mainly of goblet cells. The submucosa also contains these neurons and in addition numerous eosinophils. The muscularis is thickened by bundles of dense fibrous tissue which are continuous with the mesenteric nodules. The latter are composed of neurofibromatous structures, some also presenting myxomatous changes. Definite neurons are not demonstrable in the nodules of the mesentery.

About 1 week postpartum one of the subcutaneous nodules from the neck was removed for examination. This revealed a neurofibroma identical in structure with the neurofibromas of the mesentery. In one of these nodules the nerve fibers constituting the neoplasm were continuous with those of a normal nerve trunk in the periphery of the mass.

The patient had a normal puerperium and was discharged from the hospital in good condition without any undue symptoms. When last seen (about 2 months later) she was in a normal state of health and the subcutaneous nodules in the neck and abdominal wall were found to be greatly reduced in size.

THE DEMONSTRATION OF PLASMA PROTEINS IN THE TISSUE BY MEANS OF FLUORESCENCE MICROSCOPY. Hans P. Popper (by invitation), Chicago, Ill.

*Abstract.* The escape of plasma proteins into the interstitial tissue caused by damage to the capillaries and loss of semipermeability, with subsequent edema (serous inflammation), may occur in the loose interstitial tissue of the large parenchymatous organs. It produces malnutrition of the paren-



chymatous cells, disturbances of the structure, and opens the pathway for toxins in the tissue; if progressive, it is followed by sclerosis. The histological demonstration of serous inflammation meets with the difficulty that the plasma proteins show no microscopic differences from the tissue proteins. The observation of the spontaneous fluorescence of unstained slides of normally embedded organs, fixed in Carnoy's solution, reveals a difference between both types of proteins. The plasma proteins within the blood vessels and those escaping into the interstitial tissue are clotted and granular, and show a brown fluorescence as distinguished from the gray to blue of the tissue proteins. By staining with fluorescent dyes (fluorochromy) the difference can be exaggerated. With methyl green and thioflavine S at pH 6.0 the plasma proteins become a deeper brown color.

The serous hepatitis, as seen in coma, intoxications, infections, and especially in the first stage of parenchymatous jaundice, is characterized by wide spaces between the capillary wall and the liver cell cords which are filled with brown plasma proteins. This proves that the Disse space is not an artificial postmortem product due to shrinking or autolysis. The capillary wall is seen more distinctly in fluorescence than in the usual light. The wall of the capillaries is thicker in serous inflammation and is a sign of capillary involvement in contrast to the increased permeability. The thickening is probably due to an edema of the wall and may be followed by sclerosis of the capillaries with complete loss of permeability.

Serous myocarditis may be seen in all forms of suffocation, intoxication, and infection, but especially in acute rheumatic myocarditis and a hypertensive heart. The capillaries reveal a thickening of the wall and are surrounded by protein granules. In hypertensive heart disease where these changes are extensive, proliferation of connective tissue fibers in the edematous interstitium is visible (myocarditis serofibrosa), with consecutive scar formation due to capillary changes.

The slightest degree of serous nephritis is characterized by an escape of plasma proteins through the filtering glomerular loops (albuminuria), often combined with thickening of the wall of the loops (as in nephrosis). In the tubules the plasma proteins may be mixed with those originating from the tubular cell plasma. The next stage is the escape of proteins in the area of the reabsorbing capillary aggregations in the renal medulla. The proteins are visible between the capillaries, as seen in disturbances of bloodflow, hypertension and pyelonephritis. In the chronic stage proliferation of the connective tissue is seen as in chronic pyelonephritis, diabetes and cirrhosis of the liver. The severest stage of serous nephritis is the generalized edema of the kidney with the presence of serum proteins between the capillaries and the tubules of the cortex also. This may occur in acute glomerulonephritis and severe infections, sometimes combined with severe functional damage. It is the prodromal stage of the proliferation of connective tissue in the interstitium as seen in chronic nephritis or malignant hypertension.

**TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM.** Edith L. Potter and John M. Parrish (by invitation), Chicago, Ill.

*Abstract.* Neuroblastoma and ganglioneuroma are universally conceded to be tumors composed of cells representing different degrees in the development of primitive tissue arising originally from the neural crest of the em-

bryo. The structure varies from tumors composed entirely of completely undifferentiated cells (sympathogonia), moderately differentiated (sympathoblasts), or completely differentiated (ganglion cells) to those in which there is a mixture of all elements. Schwannomas have been found in association with chromaffin tumors, but only rarely in a patient with a neuroblastoma or a ganglioneuroma.

The case reported is that of an infant born dead 2 months prematurely in whom multiple tumors were present. The entire chain of paravertebral sympathetic ganglia was transformed into a continuous mass of tumor tissue 1 to 1.5 cm. in diameter. Fused with the inferior surface of each adrenal were lobulated circumscribed tumors, each measuring 5 by 5 by 6 cm. Connecting these across the midline was a similar tumor of approximately equal size. The wall of the urinary bladder was composed of firmer, more fibrous tumor tissue which measured 3 cm. in thickness at its greatest width. It extended posteriorly and encircled the rectum, the fibers ending at the anus. Numerous, small encapsulated masses of tumor tissue were present throughout the posterior part of the abdominal cavity. There was an extreme hypertrophy of all nerves in the body, particularly those of sympathetic origin. Multiple minute tumor nodules were present throughout the liver.

Microscopically all of the tumors show a general similarity in structure and are composed of sympathogonia, sympathoblasts, ganglion cell and nerve fibers, with irregular areas of hemorrhage, calcification and necrosis. Sympathogonia form the main portion of the adrenal tumors; ganglion cells and fibers compose the greater part of the ganglion tumors, but all types form a diffuse mixture in all tumors.

The tumor of the bladder is different from those in other locations. It consists of large masses of tissue composed of elongated nuclei and fibrillar material separated into bundles by collagen fibers. In one area groups of immature ganglion cells are present, but the greater part of the tumor consists only of Schwann cells and fibers and is characteristic of a Schwannoma.

The hypertrophy of the nerves is due to an increase in Schwann cells accompanied by local areas of excessive acellular fibril proliferation.

This case exemplifies the interrelation of neuroblastoma, ganglioneuroma and Schwannoma. The tumor is not due to activation of misplaced rests of embryonic tissue but to some condition affecting the entire sympathetic nervous system, stimulating generalized neoplastic development.

#### OBSERVATIONS ON THE VIRULENCE AND OTHER PROPERTIES OF STREPTOCOCCI ISOLATED IN STUDIES OF INFLUENZA. E. C. Rosenow, Rochester, Minn.

*Abstract.* By the use of dextrose brain broth and soft dextrose brain agar it has been possible to isolate a streptococcus from the nasopharynx in all and from the blood in about one-half of a number of cases of influenza occurring during the epidemic of 1936-1937 and 1938-1939. The different strains isolated were much alike in cultural characteristics and in virulence. Nearly all produced the alpha type of hemolysis on horse blood agar plates, a few the beta type of hemolysis. When 0.1 cc. of a 1:200 or 1:1000 dilution of the primary dextrose brain broth culture of the streptococcus and of rapidly made subcultures in this medium were injected intracerebrally into rabbits and 0.03 cc. intracerebrally into mice, severe lesions of the mucous membrane of the trachea, bronchi, and the lungs developed. The lungs became greatly

distended and revealed widespread interspersed areas of emphysema, hemorrhagic edema and bronchopneumonia. Like injections of the streptococci from patients with diseases other than those of the respiratory tract seldom caused lesions of the lungs. An antiserum was prepared in horses by repeated injections of the streptococcus isolated in previous outbreaks and whose specificity was maintained throughout in a dense suspension of glycerol (2 parts) and 25 per cent salt solution (1 part). Nearly all patients suffering from influenza have yielded an immediate erythematous-edematous reaction on intradermal injection of approximately 0.03 cc. of a 10 per cent solution of the euglobulin fraction of this serum and no flares or slighter flares to like fractions of other antistreptococcal serums, antipneumococcus serums (types I and II) and normal horse serum, injected as controls. Positive ring precipitation tests with the serum and cleared nasopharyngeal washings from patients with influenza and the influenza antistreptococcal serum were obtained in nearly all cases. Control antistreptococcal serums yielded no clouding or lesser reactions. In a number of cases of typical influenza which did not develop pneumonia the streptococcus was isolated, and particularly marked skin reactions and positive precipitation reactions with the influenza antistreptococcal serums were obtained during the initial chill and lesser reactions in many other patients during the early stages of the disease, or in what is now considered as the virus phase of influenza. These results have been obtained in each of four widely separated outbreaks. The possible relation between the streptococcus and the virus is under study.

**TISSUE RESPONSES TO RED BLOOD CELLS.** E. L. Sarason (by invitation), R. M. Thomas and M. C. Winternitz, New Haven, Conn.

*Abstract.* Injection of homologous blood or 1 per cent Kaolin into the peritoneal cavity of rabbits, gauged to produce equivalent monocytic reaction, shows both fat and iron particles within the monocytes after blood, but not after Kaolin. The participation of the red blood cells in the production of the lipid of the exudate as well as the iron correlates with discoveries in diseases of the vessel wall.

**THE ELECTRON MICROSCOPE AND ITS USE IN LOCALIZING MAGNESIUM AND CALCIUM IN TISSUE SECTIONS.** Gordon H. Scott, St. Louis, Mo.

*Abstract.* When tissues are fixed by rapid freezing and dehydrating in vacuo at  $-60^{\circ}$  C. there is but slight chance of shifts from the original location of the inorganic salts. Tissues prepared in this manner are infiltrated with paraffin without having been exposed to air, and sections cut at  $10\ \mu$ . These sections are placed on the cathode of an electron microscope and heated slowly until the salts begin to emit electrons. The electron beam is then passed through a pair of focussing magnetic fields and the resulting image thrown on a fluorescent screen. The source of electrons is easily recognizable as the treatment does not destroy topographical relations. Thus it is possible to distinguish in the image on the fluorescent screen cells, parts of tissues, and so on. Appropriate treatment of the cathode makes it possible to determine specifically the location of magnesium and of calcium. Skeletal muscle when prepared in this manner shows a distinct pattern of magnesium and calcium in the contraction bands of the fiber. Epithelium of the alimentary tract,

nerve cells and other tissues has also been examined and the magnesium and calcium localization ascertained. Further experiments designed to localize sodium and potassium are in progress.

MULTINUCLEATE GIANT CELLS WITH INCLUSION BODIES IN A FATAL CASE OF  
PRODROMAL MEASLES. K. H. Semsroth, Amsterdam, N. Y.

*Abstract.* A boy 20 months old was twice exposed to measles during 14 days. Two weeks after the first exposure acute dyspnea with respiratory death occurred. The clinical diagnosis was foreign body of the larynx, which was not corroborated at autopsy. The thymus, tonsils, hilum nodes and lymph follicles of the spleen showed numerous multinucleate giant cells of the type found to be pathognomic for measles by Warthin and others. In the epithelium of the respiratory passages degenerative changes were associated with proliferative changes. The latter consisted of the appearance of amitotic multinucleate epithelial giant cells comprising cytoplasmic inclusion bodies. Mononuclear peribronchiolitis and interlobular pulmonary edema were also present.



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## TOXOPLASMIC ENCEPHALOMYELITIS \*

### III. A NEW CASE OF GRANULOMATOUS ENCEPHALOMYELITIS DUE TO A PROTOZOON

ABNER WOLF, M.D., DAVID COWEN, M.D., AND BERYL H. PAIGE, M.D.

*(From the Neuropathology Department, College of Physicians and Surgeons,  
Columbia University, the Neuropathology Department of the  
Neurological Institute, New York, and the Department of  
Pathology of the Babies Hospital, New York City)*

In 1937 Wolf and Cowen<sup>67</sup> reported a case of parasitic encephalomyelitis in an infant. The causative agent was found to be a protozoon which on morphological grounds was identified as an Encephalitozoon. At the time, however, the possibility that the parasite might be a Toxoplasma was considered. In this paper reports of similar infections in infants by Jankû<sup>21</sup> and Torres<sup>59-61</sup> were cited as instances of the same disease. Later, a case reported by Richter<sup>49</sup> was suspected, because of its pathological picture, of being the same disease, and reexamination of his histological sections revealed the presence of parasites similar to those observed in the first 3 cases cited.<sup>68</sup> In reviewing Richter's case the identity of the causative parasite was reconsidered and it was pointed out that the information available at that time was somewhat in favor of the microorganism being Toxoplasma, rather than Encephalitozoon. This decision again was reached on morphological grounds, but it was indicated that the final evidence as to the identity of the microorganism would be procured only if it could be recovered from a future case and studied experimentally.

\* Investigation carried out under a grant from the Friedsam Foundation.  
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A recent case, which came to autopsy at the Babies Hospital in New York City in June 1938, presented an opportunity for the transmission of the infection to experimental animals. A preliminary report<sup>69</sup> of the transmission experiments establishing the identity of the causative agent as a *Toxoplasma* has been made.

*Toxoplasma* is generally regarded as a protozoan parasite, the exact classification of which is as yet undetermined. The micro-organism has been known since 1908 when it was reported independently by Splendore<sup>55, 56</sup> (Brazil) in the rabbit, and by Nicolle and Manceaux<sup>41, 42</sup> in the North African rodent, the *gondi*. In smears the parasites appear as well outlined, crescentic or curved masses of protoplasm 4–6 $\mu$  in length and 2–3 $\mu$  in width. The extremities are usually pointed, although one end may be rounded. In histological sections of fixed tissue, or under pressure, ovoid, rounded or fusiform types are more commonly seen. Each parasite contains a rounded chromatin mass situated centrally or nearer the blunter extremity. The chromatin body may appear granular or ring-like, possibly due to incomplete staining, or rod shaped or dumbbell shaped preparatory to cell division. Flagella are absent and motility has not been observed. Reproduction is by simple binary longitudinal division. In addition to the single parasites, large rounded masses of what appear to be aggregations of closely approximated parasites are also seen. Such masses are sometimes referred to as cysts although it is uncertain whether or not they possess a true cyst wall. In the cysts it is not always possible to distinguish clearly between the individual parasites which may appear to be differentiating out of a multinucleated mass of protoplasm. Such forms have been interpreted by some as indicative of reproduction by schizogenesis. It has not been possible to cultivate the parasites in artificial mediums although success has attended the use of those containing living cells.<sup>28, 51</sup>

In the past 30 years *Toxoplasma* has been described as a spontaneous infective agent in a variety of species of mammals and birds from many parts of the world. The strikingly wide geographic distribution of, and host susceptibility to these parasites is indicated by the following reports of naturally occurring toxoplasmosis: dog (Italy,<sup>35</sup> Brazil,<sup>7</sup> Germany,<sup>72</sup> France,<sup>3, 39</sup> Persia<sup>30</sup>);

rabbit (Brazil,<sup>50</sup> Senegal,<sup>4</sup> France,<sup>28</sup> Dutch East Indies,<sup>5</sup> Congo<sup>52</sup>); guinea pig (Brazil,<sup>9</sup> France,<sup>37</sup> United States<sup>32, 51</sup>); mouse (Italy,<sup>53</sup> France<sup>38</sup>); rat (Italy<sup>54</sup>); gondi, *Ctenodactylus gondi* (Tunisia<sup>42, 43</sup>); squirrel (England<sup>13</sup>); mole (Japan<sup>48</sup>); snake (London Zoological Garden<sup>47</sup>); lizard (France<sup>14</sup>); the fossa, *Cryptoprocta ferox* (London Zoological Garden<sup>47</sup>); wombat (Wellcome Bureau, London<sup>15</sup>); monkey, *Stentor senilicus* (Guiana<sup>57</sup>); baboon, *Cynocephalus* (France<sup>29</sup>); chimpanzee (France<sup>22</sup>); domestic pigeon (Brazil,<sup>7</sup> India<sup>34\*</sup>); English sparrow, *Passer domesticus*; starling, *Sturnus vulgaris*; canary, *Serinus canarius* (United States<sup>18, 19, 31, 70</sup>); siskin (Germany<sup>64</sup>); and various other birds from the same and other lands.†

The morphology of the microorganisms and the histopathology of the disease produced in the animals are often very inadequately described. Some of the animals may not have had spontaneous toxoplasmosis since they were inoculated for other purposes and may have inadvertently received injections of tissues which were *Toxoplasma*-infected. Such biological characteristics as might be studied by transmission of the infection are lacking in many instances. Most of the reports however, are sufficiently docu-

\* In these reports the parasites were not designated as *Toxoplasma* by the author, but subsequent writers<sup>33, 60</sup> have generally so regarded them.

† These include the following: Brazil<sup>2\*</sup>: white-throated seed-eater (*Sporophila albogularis*), Andean white throat (*Brachyspiza capensis*), white-bellied swallow (*Attilora cyanoleucus*), yellow finch (*Sicalis flaveola*), palm tanager (*Tanagra palmarum*), Dominican cardinal (*Paroaria larvata*), red rump tanager (*Rhamphocelus brasilius*). Brazil<sup>6</sup>: rufous-bellied thrush (*Turdus rufiventris*), blue-black grassquit (*Volatinia jacarini*), (*Adaptus etiopi*), tyrant flycatcher (*Pitangus sulphuratus*), white-crested elaenia (*Elaenia albiceps*), king vulture (*Gypagys papa*). Brazil<sup>10</sup>: tanager (*Tanagra sayaca*). Gambia<sup>60\*</sup>: African vulture (*Neophron monachus*). India<sup>1\*</sup>: sparrow. France<sup>23\*, 25, 33</sup>: Java sparrow (*Padda oryzivora*), waxbills (*Estrilda phoenicotis*), (*Lagonosticta senegala*), weaver bird (*Quelea erythrops*), fire finch (*Pyromelona francisca*), chaffinch (*Fringella coelebs*), yellow babbler (*Liothrix luteus*). London Zoological Garden<sup>47</sup>: fruit pigeon (*Carpophaga cinnina*), pied bush chat (*Pratincola caprata*). United States<sup>45\*</sup>: sparrow. United States<sup>20</sup>: catbird (*Dumetella carolinensis*), chipping sparrow (*Spizella passerina*), kingbird (*Tyrannus tyrannus*), red-eyed towhee (*Pipilo erythrophthalmus*), song sparrow (*Melospiza melodia*), swamp sparrow (*Melospiza georgiana*), Baltimore oriole (*Icterus galbula*), cowbird (*Molothrus ater*), Savannah sparrow (*Passerculus savanna*). United States<sup>71</sup>: house finch (*Carpodacus mexicanus frontalis*). Argentina<sup>60</sup>: canary (*Serinus canarius*). Japan<sup>62, 63</sup>: white-eye (*Zosterops palpebrosa peguensis*), paddybirds (*Munia malacca*, *Munia maja*, *Munia atricapilla*, *Munia topela*, *Plageus baya*, *Aidemosyne malabarica*), bamboo finch (*Erythrura prasina*), Java sparrow (*Oryzornis oryzivora*). Italy<sup>17</sup>: European tree sparrow (*Passer montanus*), Italian house sparrow (*Passer italiae*). Germany<sup>44</sup>: English sparrow (*Passer domesticus*), green finch (*Ligurinus chloris*), linnet (*Cannabina linota*).



mented and support the impression of the wide distribution of the infection.

Following the usage of Nicolle and Manceaux, who named their parasite *Toxoplasma gondii*, many subsequent observers have referred to each newly reported strain as *Toxoplasma cuniculi*, *T. musculi*, *T. columbae*, and so on, according to the host in which it was discovered. As a number of authors<sup>12, 68</sup> have pointed out, however, there is no morphological or other means of distinguishing between the various named forms of the parasite, and it may well be that they represent a single species capable of infecting many hosts. This low host specificity has been demonstrated experimentally on many occasions by inoculation of *Toxoplasma*-infected tissue into heterologous animal species. Thus, *T. gondii*<sup>12, 24, 43</sup> has been found pathogenic for the mouse, guinea pig, pigeon, rabbit, mole, shrew, dog, Java sparrow and cat; *T. cuniculi*<sup>28</sup> for the guinea pig, mouse, pigeon, chick, sparrow and other small birds; *T. canis*<sup>39, 40</sup> for the rabbit, guinea pig, mouse, sparrow and pigeon; *T. caviae*<sup>9, 51</sup> for the pigeon, mouse, rabbit, chick and chicken. Nothing is as yet known of the natural mode of transmission of the infection in animals or of the existence of intermediate vectors. In naturally infected animals the parasites have commonly been found free or in leukocytes in inflammatory parenchymal lesions and exudates in a variety of tissues, notably the brain, spinal cord, spleen, lung and liver. They have also been observed occasionally in the blood, bone marrow, intestinal mucosa, lymph nodes, heart, kidney, pancreas, omentum, mesentery and pleural exudate. The pathological changes in spontaneously infected animals have in most instances not been thoroughly described. The lesions most commonly encountered are small focal inflammatory nodules, with or without necrosis, in the spleen, lung and liver. The infiltrating cells are predominantly lymphocytes and large mononuclears. Comparable changes have been noted in the brain although there is little or no reaction to the presence of the parasites in this organ in some instances. The intestine may show ulceration. The kidney generally shows no pathological changes. The lesions in experimental toxoplasmosis, as described in the literature, and as we were able to observe them in rabbits and mice inoculated with a strain of *Toxoplasma* of animal origin<sup>51</sup> from the Rockefeller Institute of New York,

kindly furnished us by Drs. Sabin and Olitsky, may be briefly summarized. Intracerebral inoculation of rabbits results in chronic inflammatory foci in the leptomeninges, parenchyma, ventricular walls and choroid plexuses. The infection may spread down to the spinal cord. The lesions contain lymphocytes and mononuclear cells with fibrin and polymorphonuclear leukocytes when necrosis occurs. Plasma cells are common in the chronic lesions. Productive changes, such as multiplication of leptomeningeal cells, endothelial proliferation with production of new capillaries, formation of small granulomas of capillary origin, and proliferation of microglia and fibroblasts, are of frequent occurrence. The parasites occur free, in cysts, and intracellularly, in leptomeningeal cells, large mononuclear cells, endothelial cells of capillaries, epithelioid cells of the granulomas, phagocytes, ependymal and choroid epithelial elements, and rarely in nerve cells within the focal lesions. They are most numerous in the necrotic foci, although they may occur in considerable numbers about involved blood vessels, spreading into the parenchyma without much initial reaction. There are focal inflammatory and, at times, necrotizing and productive lesions in other organs. The lungs, liver and spleen, and less commonly the heart, adrenals, kidneys, intestines and lymph nodes are affected. Parasites are present in these lesions. Combined intracerebral and intraperitoneal inoculation of mice results in essentially the same picture with an associated peritonitis. Parasites may be found free and in large mononuclear cells in the peritoneal exudate. Infection also develops after inoculation by other routes with widespread dissemination and variations in the intensity and localization of the lesions in various organs.

The following is a description of the clinical and pathological changes observed in an infant from whom *Toxoplasma* was recovered. In view of the rarity of this condition a complete report of the case is presented.

#### REPORT OF CASE

*Clinical History:* C.D. (Babies Hospital, New York, No. 552107), a white male infant, was delivered at term by Cesarean section on May 23, 1938.

Both parents were in good health and had always resided in or near New York City. No history of contact of the parents with rabbits or other animals could be elicited. The mother had never eaten rabbit meat. The apartments

in which she had resided for several years before admission to the hospital were free of mice and rats. No pets, including birds, were kept in the home. The father was 32 years of age. The mother, a primipara 31 years of age, had been under continuous observation in the hospital for the last 3 months of her pregnancy as a suspected case of placenta praevia. During this period her temperature, pulse, respiration and blood pressure remained within normal limits. Her blood Wassermann test was negative, and the course of pregnancy was uneventful except for slight vaginal bleeding during the last months. Several days before the expected date of delivery there was spontaneous rupture of the membranes with a profuse discharge of yellowish fluid. During pelvic examination a large amount of thin meconium escaped. In view of the placenta praevia, it was decided to deliver the child by Cesarean section. This was performed under nitrous oxide-ether anesthesia. Recovery from the operation was uncomplicated. The placenta and membranes were unfortunately not kept for examination.

At birth the infant was moderately asphyxiated but respiration was described as spontaneous. Crying was not vigorous. The infant weighed 3050 gm. and measured 50 cm. in length. The head measured 33.5 cm. in circumference, the chest 31 cm., and the abdomen 33 cm. On the 3rd day of life the infant had a right-sided convulsive seizure, lasting 3-4 minutes, with jerking of the hands, twisting of the mouth, and rolling of the eyes to the right. During the attack the respirations were shallow and rapid. There was no rigidity of the neck or bulging of the anterior fontanelle. The body temperature remained normal. Following the convulsion the infant was apathetic and respirations were irregular, shallow and rapid. A left-sided Horner's syndrome was observed. No gross hemorrhages were noted in the fundi. There was a normal withdrawal reaction to pin prick. The tendon reflexes were absent in the legs and hypoactive in the arms.

During the next few days of life the child's condition improved somewhat. There were repeated convulsive seizures but these gradually became less frequent. Feedings were taken fairly well, although in general the infant was drowsy and lethargic. By the 2nd week convulsions ceased, although weakness and a left enophthalmos persisted. A roentgenogram of the lumbosacral spine at this time showed no abnormalities. Lumbar puncture yielded bloody fluid but the relatively uncontaminated portions of the fluid appeared xanthochromic.

Physical examination on the 17th day of life showed retraction and turning of the head toward the right. The eyes deviated to the right and at times there was nystagmus toward the right. The upper extremities were spastic and the hands clenched, but the tendon reflexes were not increased. No thoracic breathing could be made out. The abdominal reflexes were absent, but sluggish cremasteric responses could be obtained. The lower extremities were withdrawn on painful stimulation. Babinski reflexes were not elicited. Response to pin prick was definitely less marked below the neck than on the face and scalp. The spleen was just palpable. The bladder was distended and there was dribbling of urine. The fontanelles were not tense nor was there any separation of the cranial sutures. It was felt that the clinical signs pointed to both spinal cord and cerebral injuries with the more severe injury in the spinal cord. The patient was discharged from the hospital at the age of 18 days.

At home there was at first no change in the infant's condition. On the 22nd day of life, however, the mother noticed a seizure in which the child's arms became stiff and remained straight and stiff by his sides. Following this episode he seemed weaker. On the 26th day vomiting began and although the mother diluted the formula and fed it in small amounts, the feedings were not retained. For this reason the infant was readmitted to the hospital after having been at home for 10 days.

As contrasted with examination several days previously, the infant's condition was poor. Respiration was quite irregular and labored, and appeared to be almost entirely diaphragmatic in type. There was moderate cyanosis and a grayish tint to the skin. No spontaneous movements below the neck were observed. The arms were pronated and held in extension at the elbows, and the fingers were kept flexed. Attempts to elicit the deep reflexes in the lower extremities resulted in flexion of the thighs and legs, and dorsiflexion of the feet. The abdominal reflexes were absent. There appeared to be insensitivity to pain below the neck. Both liver and spleen were palpable but thought by the examiner to be of normal size.

*Laboratory Data:* Examination of the urine on June 20, 1938 showed a trace of albumin, no sugar, and microscopic examination showed 6-30 polymorphonuclears per high power field with occasional erythrocytes. A blood count on June 20th showed erythrocytes 5,970,000 per cmm., total leukocyte count 8000 per cmm.; polymorphonuclear leukocytes 61 per cent, lymphocytes 33 per cent, eosinophils 5 per cent, and monocytes 1 per cent. Examination of the spinal fluid on June 21st showed a pressure of 140 mm. of water. Respiratory oscillations were present but there was no response to compression of the jugular vein on either side. Three cc. of slightly cloudy, distinctly xanthochromic fluid were removed. This clotted promptly and gave a 4 plus Pandy reaction. The benzidine test was negative. Microscopic study of the fluid was not made. A blood Kahn test on June 23rd was negative.

*Terminal Clinical Observations:* In view of the suspected involvement of the cervical cord with subarachnoid 'block,' a cisternal puncture was attempted. About 3 drops of rather viscid, clear, slightly xanthochromic cerebrospinal fluid were obtained which gave a positive benzidine test and a 4 plus Pandy reaction. About 0.1 cc. of lipiodol was injected. Fluoroscopy and roentgenographic examination of the spine at intervals following this procedure showed all of the lipiodol to remain above the level of the second cervical vertebra, suggesting a 'block' at this point. During the last few days of life the infant's temperature fluctuated between 96° and 101.2° F. The cry was weak and respirations were of the Cheyne-Stokes type.

Examination of the fundi revealed an irregular reddish brown area in each macular region about 1 disc-diameter in size on the right, and somewhat smaller on the left. These changes were interpreted as resulting from hemorrhage.

On June 23, 1938, the infant became markedly cyanotic with rapid shallow respiration. At death the baby was 31 days of age. The clinical diagnosis was multiple injuries of the central nervous system with compression of the cervical spinal cord by hematoma.

## AUTOPSY REPORT

Postmortem examination was performed 4½ hours after death and was limited to examination of the brain, spinal cord and posterior hemisphere of the right eye.

The surfaces of the cerebral hemispheres presented numerous focal lesions (Fig. 1) varying from a few mm. to approximately 2 cm. in diameter. In these areas the cortex was depressed, pitted and discolored yellow. This tissue was in most instances considerably softer than that of the surrounding unaffected gyri, and the overlying leptomeninges were thickened and grayish yellow in color. The most prominent lesions involved portions of the superior and middle frontal gyri, the frontal and temporal poles, the precentral and postcentral gyri, the superior parietal lobule and the lateral occipital gyri bilaterally. Most of the gyri on the medial and ventral surfaces showed similar focal lesions. The cerebellum and brain stem appeared normal externally. The leptomeninges about the basal cisterns were slightly grayish and opaque.

Coronal sections of the left cerebral hemisphere showed that the superficial lesions consisted of well demarcated zones of cortical softening (Fig. 2). The center of each was slightly sunken, finely cystic, and yellowish or grayish yellow in color, while its margin was sharply outlined, grayish and more prominent. The larger lesions extended from the cortex into the subcortical white matter, and at times into the centrum ovale. On incising some of the softened areas the knife passed through gritty material. A large cyst, 5 cm. in length anteroposteriorly, 1.5 cm. in diameter in its greatest cross section, and lined by necrotic discolored parenchyma, was present in the white matter inferomedial to the atrium and occipital horn of the left lateral ventricle.

Single and conglomerate foci of softening similar to those in the cortex involved portions of the island of Reil, and lenticular and caudate nuclei. The thalamus and hypothalamus appeared grossly unaffected.

The changes in the right cerebral hemisphere were much the same as those in the left, except for the absence of large cysts.

The lateral and third ventricles were slightly dilated. Their walls were smooth and glistening, except in the inferomedial

angles of the lateral ventricles where there were areas of yellowish discoloration denuded of ependyma.

Section of the cerebellum revealed no gross abnormalities, while the brain stem contained numerous lesions. There were numbers of soft, irregular, yellowish white areas in the midbrain, each bordered by a grayish translucent marginal band. The largest area was located in the right cerebral peduncle and measured 5 by 2 mm. in cross section. The others were present in the oculomotor nuclei and ventral portions of the red nuclei. The aqueduct of Sylvius was of normal size. In the cephalic portion of the pons, at the junction of the tegmentum and reticular area, was an ovoid, translucent grayish area 5 by 3 mm. in cross section, traversed by fine yellow, curving and interlacing lines (Fig. 7). Small, cream colored, sharply margined lesions, pin-point in size, were scattered through the reticular zone and in the regions of both fifth nerve nuclei. In the cephalic portion of the medulla two dull white areas with gray centers and scalloped edges were found in the restiform bodies and fifth nerve nuclei bilaterally, the right measuring 4 mm. and the left 2 mm. in diameter. At their margins white pin-point nodules were seen. The rest of this portion of the medulla was glassy in appearance and showed slight obscuration of its markings. In the midmedulla there were numerous lesions similar to those described in the midbrain. Many of those on the right side were softened centrally. The largest lesion was 10 by 5 mm. in cross section, oval, sharply margined, grayish white in color, and involved almost the entire left side of the medulla at this level. At the junction of the middle and lower thirds of the medulla a syrinx measuring 4 mm. in length and 1 mm. in transverse diameter was present. It was lined by a narrow band of yellowish and gray translucent tissue, widest at the lateral border of the cavity. The right half of the lower medulla was soft to palpation and its architectural markings were obscured by gray and cream colored mottling.

The cervical segments of the spinal cord were markedly swollen, the greatest swelling occurring in the region of the cervical enlargement, which was firm and almost twice its normal size. The leptomeninges over its cephalic portion were slightly clouded. The first three segments of the thoracic cord were shrunken to about one-half of their normal diameter, and were softer than the ad-

jacent parenchyma. The leptomeninges over them were opaque and yellowish. There was a similar change in the seventh, eighth and ninth thoracic segments and overlying pia-arachnoid. The lowest thoracic segment was slightly swollen. The lumbosacral segments of the spinal cord, the nerve roots and the cauda equina appeared normal. Except in the regions where they were specifically noted as abnormal, the leptomeninges were thin and translucent. On section of the swollen cervical cord the parenchyma was observed to be translucent and gray, and the line of demarcation between gray and white matter was obliterated (Fig. 3). Section of the shrunken upper segments of the thoracic cord (Fig. 4) revealed that their normal markings were obscured. The tissue was gray and translucent and in it there were a number of punctate yellow and brownish nodules. In the seventh to ninth thoracic segments (Fig. 5) an area of grayish and light brown glistening tissue measuring 4 by 2 mm. in cross section was present in the central portion of the parenchyma obscuring all the gray horns. At the margins of this zone the tissue was studded by yellow punctate nodules 1-2 mm. in diameter. In the upper lumbar segments (Fig. 6) the outlines of the ventral horns were well preserved. The posterior horns, however, were obliterated by grayish tissue which passed into the posterior columns and parts of the lateral columns as well. Yellowish nodules, 1 mm. or less in diameter, were occasionally seen in the white matter.

#### HISTOLOGICAL EXAMINATION

*Leptomeninges of Cerebrum:* Over the cortical lesions the leptomeninges were markedly edematous and congested. There was moderate or intense infiltration by lymphocytes, plasma cells, mononuclear leukocytes, lipid laden phagocytes and eosinophils (Fig. 11). In some areas the last were almost as numerous as the plasma cells. Where the parenchymal changes were most severe the leptomeningeal exudate was directly continuous with that in the cortex, obliterating the line of demarcation between pia-arachnoid and brain. There was a multiplication of leptomeningeal cells. None of the leptomeningeal vessels was occluded, but there was endothelial hyperplasia in all the capillaries, many of the arterioles and venules, and some of the small arteries and veins.

Over the unaffected gyri the leptomeninges were somewhat edematous and occasionally infiltrated by small numbers of mononuclear leukocytes and lymphocytes.

*Cerebral Cortex:* There was a complete loss of cortical architecture and a total destruction of all neural and glial elements in the large zones of necrosis (Fig. 8). These were occupied by a mass of eosinophilic and nuclear débris in which only a few vessels were still partially preserved. Their walls were permeated and surrounded by fibrin. Toward the margins of these areas of total degeneration, polymorphonuclear leukocytes, lymphocytes, plasma cells, lipoid laden phagocytes and eosinophils were present in moderate numbers. Frequently they were concentrated in the walls and perivascular spaces of blood vessels. The parenchyma was intensely edematous and showed a considerable loss of neural and glial elements, moderate hyperplasia of capillaries, and considerable astrocytosis. The majority of the astrocytes showed intense clasmatodendrosis. Beyond the marginal zone of reaction the parenchyma was edematous and studded by numerous milium granulomas (to be described in detail below). Between the granulomas in the gray matter there was a moderate diffuse loss of ganglion cells, and many of the remaining cells were either swollen and vacuolated or pyknotic. The oligodendroglia were swollen and the astrocytes hypertrophied and fibrillary.

In some of the severely necrotic areas cyst formation occurred. Within the cavities lipoid laden phagocytes lay in a meshwork of hyperplastic capillaries, the walls of which were infiltrated by small numbers of plasma cells, lymphocytes and mononuclear leukocytes. Fibroblasts derived from the capillary walls were present. There was multiplication of microglia cells at the margins of the cavities and of the necrotic zones. They gave rise to numerous rod cells and transition forms of lipoid laden phagocytes. In relation to the cysts, astrocytosis was usually more prominent, the cells consisting of plump astrocytes, some of which were binucleated or multinucleated.

A striking feature of the process was the marked calcification of necrotic material in some areas (Fig. 9). This involved either the majority of the cortical layers, resulting in a broad band of calcification, or consisted of scattered calcific material, such as



was seen at the margins of the cysts. The bands of calcification often stopped abruptly. The calcium occurring in coarse granules was deposited in clusters or diffusely. Considerable numbers of calcified cells (Fig. 15), the majority of which appeared to be nerve cells, were identified. The margins of these were sharp and the nuclei remained unencrusted. Short undulating bands of calcium of varying thickness had the appearance of encrusted dendrites or axones.

The cortex adjacent to these foci of severe cortical necrosis, inflammation and calcification was often surprisingly well preserved. Single granulomas were occasionally encountered in these areas, most frequently in the zonal or pyramidal layers (Fig. 10). The cortical architecture was normal but there was a diffuse edema and a slight diffuse loss of ganglion cells. The remaining neural elements had undergone complete chromatolysis, and some were swollen, vacuolated or pyknotic. No myelin sheaths were present in the gray matter, but moderate numbers of unchanged axones were observed. There was a mild generalized astrocytosis with thickening of the external glial membrane near the focal lesions.

*Cerebral White Matter:* Where the cortical lesions were most severe, inflammation and necrosis extended into the subcortical white matter and in places into the centrum ovale. Here amorphous debris was found in place of normal structures, and at its margin was a band of intensely edematous, partially degenerated white matter, moderately infiltrated by cells similar to those seen in the cortex. The capillaries in this zone were hyperplastic and there was a considerable astrocytosis, many of the astrocytes being partially degenerated. Numerous miliary granulomas were present at the margins of the zones of infiltration and in the relatively intact white matter subjacent to purely cortical lesions. The oligodendroglia were markedly swollen. Small numbers of myelin sheaths were seen in the subcortical white matter and centrum ovale. They were widely separated, but they, as well as the axones, appeared normal. The subependymal tissue about the lateral ventricles showed a marked astrocytosis. This broadened subependymal glial mat was mildly infiltrated by plasma cells and lymphocytes. The infiltration varied in intensity and where it was greatest granulomas were present. Small deposits of calcium

were occasionally seen. The walls of the lateral ventricles were denuded of ependyma at some points.

*Corpus Striatum:* There were large areas of necrosis involving the globus pallidus, putamen and caudate nucleus. Centrally these were filled with eosinophilic debris and degenerating lymphocytes, mononuclear leukocytes and polymorphonuclear leukocytes. Focal collections of such cells clustered about persisting capillaries. The degeneration passed into the adjacent internal capsule and centrum ovale. Where it reached the surface of the caudate nucleus the wall of the lateral ventricle was completely denuded of ependyma except in the lateral angle (Fig. 13). In the superficial periventricular necrotic material there were small numbers of degenerating mononuclear leukocytes and occasional clusters of calcium granules. At the margins of the totally degenerated areas the inflammatory, glial and vascular changes were similar to those described in the cortex. Granulomas in moderate numbers were scattered through the rest of this region. The nerve cells in the preserved areas showed complete chromatolysis and occasional vacuolization. Most of the myelin sheaths, not directly in the zones of necrosis, were well preserved.

*Thalamus and Hypothalamus:* The nerve cells in the thalamus were well preserved and contained a normal amount of Nissl substance. Numerous granulomas were scattered through the parenchyma, and some were present in the thickened subependymal glial mat in the wall of the third ventricle. The ependyma lining the latter was well preserved, although there were numerous ependymal granulations.

*Cerebellum:* The leptomeninges over the folia were slightly congested and contained occasional lymphocytes and monocytes. There were no abnormal changes observed within the folia proper. Occasional granulomas were found in the central white matter of the cerebellum and subependymal tissue of the wall of the fourth ventricle.

*Midbrain:* The leptomeninges were congested and contained small numbers of mononuclear leukocytes. The aqueduct of Sylvius was slightly reduced in size due to the presence of many flat ependymal granulations. Numerous granulomas similar to those in the cerebrum were scattered in the substantia nigra, cerebral peduncles, colliculi and periaqueductal region. A group

of large nerve cells in one colliculus was partially calcified. The rest of the nerve cells in the nuclei at this level showed no abnormal changes, and the axones were well preserved. The myelin sheaths appeared normal. There was a slight astrocytosis about the granulomas.

*Pons:* There were many more granulomas in this portion of the brain stem than in the midbrain, and they occurred in both the reticular and the tegmental regions, as well as in the middle cerebellar peduncles. A large area of diffuse infiltration was encountered in the midline of the dorsal half of the reticular zone. Here the tissue was quite edematous, showed mild astrocytosis, and was moderately infiltrated by lymphocytes, plasma cells, mononuclear leukocytes and occasional eosinophils. Some of the vessels showed mild endothelial hyperplasia and infiltration of their walls. The nerve cells were fairly well preserved. In foci within the zone of inflammation the process was more intense. Here there was a concentration of infiltrating cells with satellitosis and varying degrees of degeneration in some of the ganglion cells. In these foci capillary hyperplasia and microglial proliferation were also seen. Granulomas were concentrated near the margins of the infiltrated zone and involved the nearby pyramidal tracts. There were small numbers of ependymal granulations in the floor of the fourth ventricle and the subependymal glial mat was thickened and contained granulomas. The leptomeningeal changes were similar to those about the midbrain.

*Medulla:* In the cephalic portion of the medulla there were disseminated miliary granulomas. In the ventral half of the mid-medulla diffuse infiltration and partial degeneration were present. In addition to infiltrating cells similar to those described elsewhere, there were small numbers of lipoid laden phagocytes. The exudate showed focal and perivascular concentration. All of the neural and glial elements had disappeared. At the margins of the necrotic and inflammatory zone were vascular hyperplasia and a concentration of granulomas. Degeneration and inflammation were even more extensive in the caudal portion of the medulla where only a narrow band of relatively well preserved tissue containing numerous granulomas was present at the periphery. A large central cavity was present in the necrotic zone. Most of the neural elements had disappeared in the degenerated area and only

a few were preserved in the nuclei at its edges. Axones and myelin sheaths were well preserved in the marginal tissue but had disappeared elsewhere.

*Choroid Plexus:* Especially in the lateral ventricles near the glomus, there was a moderate amount of exudate between the villi of the choroid plexus. This was composed of mononuclear leukocytes, lymphocytes, and occasional polymorphonuclear leukocytes and strands of fibrin. The choroid tufts were hyperemic and their central connective tissue cores edematous. Eosinophils, scattered in small numbers beneath the choroid epithelium, were present in nearly every villus. There were fewer lymphocytes and polymorphonuclear leukocytes and these were usually found deeper in the core of the tuft. Occasionally there was a more cellular inflammatory focus, and over this area the choroid epithelium had disappeared in places.

*Spinal Cord: Upper Cervical Region:* The spinal roots showed no abnormal changes. The leptomeninges were mildly infiltrated by lymphocytes, mononuclear leukocytes, polymorphonuclear leukocytes and eosinophils. This mild infiltration passed into the dorsal medial septum and showed focal concentration over the more severe parenchymal lesions where it was associated with hyperplasia of leptomeningeal cells. The leptomeningeal vessels were patent although some of the smaller ones showed endothelial and adventitial hyperplasia. The most marked parenchymal changes occurred in the columns of Goll and were similar to those in the lower medulla, except for the absence of a syrinx. There was marginal astrocytosis and granulomas were present in the columns of Burdach. A small number of granulomas was scattered through the remainder of the white matter. There was generalized edema, most marked in the anterior horns where the nerve cells were pale staining and a few partially degenerated. Polymorphonuclear leukocytes and lymphocytes in moderate numbers were present here (Fig. 14). The central canal was distended by amorphous material in which polymorphonuclear leukocytes, lymphocytes and mononuclear leukocytes were seen. There was a total loss of myelin sheaths and axones in the posterior columns. At the margins of the degenerated zones swollen fragmented myelin sheaths were seen and phagocytosis of myelin fragments by large mononuclear cells occurred.

*Cervical Enlargement:* The spinal cord was considerably swollen at this level due to intense edema. The posterior columns showed even greater degeneration than in the preceding level with extension of necrosis and inflammation into the posterior horns and parts of each lateral column. Most of the nerve cells in the posterior horns and the medial portions of the anterior horns had either disappeared or showed marked degeneration. The remainder of the nerve cells had undergone complete chromatolysis. Granulomas were present, not only in the white matter at this level but also in the anterior horns. There was a loss of myelin sheaths in the posterior and lateral columns and a mild diffuse loss in the anterior columns. The other changes were similar to those at the preceding level.

*Upper and Lower Thoracic Levels:* These corresponded to the macroscopically shrunken regions. There was almost complete necrosis of the parenchyma which was collapsed and devoid of architectural markings. The leptomeningeal infiltration in these areas contained a greater number of plasma cells. In places the exudate passed directly into the parenchyma so that the line of demarcation between pia-arachnoid and cord proper was often lost. Granulation tissue invaded the posterior columns from the leptomeninges. Almost all of the remaining tissue consisted of cellular debris and occasional hyperplastic capillaries. All the neural and neuroglial elements had disappeared. In a few strips of partially preserved marginal white matter, mild inflammatory and productive changes, fragments of degenerated myelin, and large clusters of lipid laden phagocytes were seen.

*Lumbar Cord:* In the upper lumbar segments there was widespread necrosis of the posterior columns, dorsal portions of the lateral columns, posterior horns, parts of the anterior horns, and central gray matter and commissures (Fig. 18). The changes in these areas were similar to, but less intense than, those in the severely degenerated areas of the thoracic cord. In the lower lumbar segments and in the sacral cord there was a mild leptomeningitis. Numerous miliary granulomas were scattered throughout the parenchyma, especially in the white matter (Fig. 17). The majority of the nerve cells were normal. The descending tracts showed degeneration, while the ascending tracts were well preserved.

*Granulomas:* Each of the granulomas referred to above was composed of large polygonal, round, stellate or irregularly shaped cells (Fig. 12). These had abundant eosinophilic, homogeneous or coarsely granular cytoplasm. Their nuclei were spherical, oval or irregular, stained deeply and contained a moderate amount of coarsely granular chromatin. In some granulomas few, if any, infiltrating cells were seen. In others, lymphocytes, mononuclear leukocytes and occasional eosinophils or plasma cells were distributed among the outer epithelioid cells. Hyperplastic capillaries were often seen at the margins of these lesions or embedded in them. Their plump endothelial cells seemed to be the precursors of the epithelioid cells of the granulomas. No giant cells or any central necrosis of the lesions were encountered. In the gray matter, well preserved nerve cells were frequently found at the margins of the granulomas. Myelin sheaths were deflected at their margins or often passed directly through the granulomas unchanged. Small numbers of axones were similarly affected. There was a moderate astrocytosis at the margins of the lesions in some areas (Fig. 16), and occasionally persisting astrocytes mingled with the outer epithelioid cells.

*Right Eye:* One portion of the retina (Fig. 19) showed edema of all its layers and distortion of the stratum opticum, ganglionic, and inner molecular layers. The capillaries in this zone had undergone endothelial hyperplasia. Many nerve cells in the ganglionic layer were partially degenerated. The layer of rods and cones was markedly necrotic. The pigmented layer was partially disrupted and the destruction of its cells gave rise to much extracellular pigment. The choroid was congested, showed capillary hyperplasia, and was infiltrated by plasma cells, lymphocytes and eosinophils. The leptomeningeal sheath of the optic nerve showed a similar mild infiltration.

*Microorganisms:* Parasites (Figs. 20-27) were present in the lesions of the central nervous system and eyes, and occurred in two forms. They were commonly seen as single bodies, ovoid, oval, pyriform or rounded in shape, either free in the tissue or within the cytoplasm of cells. In material fixed in 10 per cent formalin, refixed in Zenker's solution, and embedded and cut in paraffin, in sections 4-5 $\mu$  in thickness, the majority of the organisms measured 2-3 $\mu$  in length, and 1.5-2 $\mu$  in width. More fell below this

range than rose above it. Each of the parasites was distinctly outlined and had a more deeply staining margin. A round, oval or band-like chromatin mass, the largest of which was one-third the size of the corpuscle, was present at or near one pole. Rarely the microorganisms were quite slender and lunate or scimitar-like in shape, and in these, lunate or comma shaped as well as band-like chromatin masses were more frequent. Two chromatin masses were occasionally encountered. One might be smaller and situated at the opposite pole of the cell. More often they were equal in size, symmetrically placed with their long axes parallel, and the parasites containing them probably represented dividing forms. The edges of the chromatin bodies were sharp and usually stained more deeply than their central portions. The cytoplasm at their margins was often lighter staining. No rod-like chromatin body (kinetoplast) was present, nor could a polar capsule or filament be identified. No budding was seen. The staining reactions were similar to those of the microorganism described in our 1st case.<sup>67</sup>

The intracellular parasites were present either singly or in considerable numbers, usually in mononuclear cells, often in epithelioid cells of the granulomas, less frequently in polymorphonuclear leukocytes and rarely in the endothelial cells of capillaries. None was found in nerve cells or eosinophils. The intracellular microorganisms lay in the cytoplasm of the parasitized cell, sometimes within a vacuole. Such cells frequently showed degeneration varying from swelling and vacuolization of the cytoplasm to extrusion of the nucleus, disruption of the cytoplasm and liberation of the microorganisms. Often the parasitized cells were surprisingly well preserved. The extracellular parasites showed varying degrees of pallor and degeneration in the necrotic areas of the parenchyma, but were well preserved elsewhere. In the areas of calcification some of the single calcific granules and a few of the clusters suggested encrusted microorganisms. The shape of the granule and an unstained area corresponding to the chromatin body were the identifying features.

The parasites were also found in compact, round or oval clusters which varied in size from 8 by  $8.5\mu$  to 16 by  $19.5\mu$ . The organisms in these masses were closely packed, often smaller than the free corpuscles, and on close inspection their individual outlines were

easily discernible in thin sections. In some clusters, however, the cytoplasm seemed to form a continuous syncytial mass, although this was not common. Such compact groups of parasites had the appearance of cysts, but they may simply have represented cells which had lost their nuclei and were completely occupied by microorganisms. Rarely a flattened nucleus was present at the margin of the cluster. At the margins of the cysts a matrix less dense than the cytoplasm of the parasites was present in the spaces between them. An outer membrane appeared to envelop the cluster. This was sharply outlined, refractile, but not doubly contoured, and as was noted in the description of our 1st case, may represent the denser appearing edge of the matrix. The majority of the cysts lay free in the tissue. Cysts were more often encountered in comparatively normal marginal tissue at the edges of the parenchymal lesions, particularly in the retina.

The cysts and individual organisms were never present in normal tissue distant from the lesions. They were found in small numbers in the leptomeningeal exudate, and in great numbers in the parenchymal lesions of the brain and spinal cord. They were occasionally seen in the ventricular exudate and in that of the spinal canal. Only a few were encountered in the choroid plexus, walls of the blood vessels and between ependymal cells. They were seen once within the lumen of a spinal leptomeningeal arteriole.

No other organisms were detected in any of the sections stained by a variety of methods.

#### SUMMARY OF CASE

A white male infant, delivered at full term by Cesarean section, became ill at 3 days of age and developed convulsive seizures, disturbances in respiration, and symptoms of involvement of the spinal cord with subarachnoid 'block' in the cervical region. Terminally, irregular reddish brown areas were observed ophthalmoscopically in each macular region. The infant died at the age of 31 days. Autopsy revealed a widespread encephalomyelitis characterized by multiple focal areas of inflammation and necrosis, and disseminated miliary granulomas. There was localized leptomeningitis in relation to the superficial parenchymal areas of inflammation. Cystic degeneration occurred in some of the lesions while others, especially those in the cerebral cortex, showed a dis-



tinct tendency to become calcified. The inflammation and degeneration in the spinal cord resulted in a marked swelling of the lower cervical segments, sufficient to account for the subarachnoid 'block' observed during life. The right eye contained a localized zone of chorioretinitis. A protozoan parasite was present, often in great numbers, in the leptomeningeal and parenchymal exudates, in the granulomas and in the lesions of the choroid and retina.

### DISCUSSION

The case reported above is the 5th recorded instance of a new disease entity, a form of granulomatous encephalomyelitis of infants. The clinical history resembles that of our 1st case in the occurrence of symptoms soon after birth, the manifestations of diffuse involvement of the central nervous system, the evidence of focal chorioretinitis, the short course and the fatal outcome. That the 2 cases are instances of a single disease is further indicated by the similar pathological changes: a widespread encephalomyelitis characterized by inflammation, necrosis and miliary granulomas, focal leptomeningitis and localized chorioretinitis. As has been pointed out elsewhere,<sup>67, 68</sup> the resemblance of the pathological changes described in 3 infants by Jankû,<sup>21</sup> Torres,<sup>59</sup> and Richter<sup>49</sup> to those in our 1st patient makes it evident that these cases are instances of the same disease.

The lesions in each of the 5 affected infants contain a protozoan parasite showing the same morphological characteristics. In sections the microorganisms are oval, ovoid or pyriform, 2-3 $\mu$  long and 1-2 $\mu$  wide, and contain deeply staining chromatin bodies which are usually spherical and polar in position. This microorganism, as seen in our 1st case, was considered to be an *Encephalitozoon* because of its resemblance to that parasite in sections and because the characteristic lesion that the latter produces in the central nervous system is a miliary granuloma similar to that found in this human disease. *Encephalitozoon* corresponds in size and shape to the causative parasite of this disease, is frequently intracellular, and occurs regularly in the form of similar cysts. Although Torres<sup>59, 60, 61</sup> favored the identification of his microorganism as an *Encephalitozoon* and named it *Encephalitozoon chagasi*, he also entertained the possibility that it might be a *Toxoplasma*. Later, Levaditi,<sup>26</sup> in discussing the identity of

the parasites in the cases of Jankû and Torres, pointed out their similarity to *Encephalitozoon* and to *Toxoplasma cuniculi*. He referred to resemblances in the pathology of these cases to experimental toxoplasmic infection in rabbits, and favored the identification of the microorganisms in the human cases with *Toxoplasma*. It was noted in our first report that the lesions in the central nervous system show a resemblance to those in experimental toxoplasmosis in the rabbit: diffuse necrotizing and inflammatory lesions of the cortex and basal ganglia at times associated with miliary granulomas which do not become necrotic centrally and which are disseminated widely throughout the brain and spinal cord. This was discounted, however, for two reasons: (1) the microorganism of the human disease, as seen in sections, did not have the morphological characteristics commonly described for *Toxoplasma* — lunate shape, larger size,  $4-6\mu$  by  $2-3\mu$ , and central chromatin body; and (2) since the human infection was not transmitted to animals, an experimental infection produced by it could not be directly compared with experimental toxoplasmosis. During a restudy<sup>68</sup> of Richter's case in which a protozoon similar to that in our 1st case was identified, an opportunity arose to make some observations on the morphology and biological characteristics of a strain of *Toxoplasma* recovered from a guinea pig at the Rockefeller Institute. It was found that in sections of lesions of the brain and other organs in the rabbit and mouse, this *Toxoplasma* was almost identical in appearance with the parasite in our 1st case and in Richter's case. In smears, however, it had the more commonly described appearance of *Toxoplasma* given above. It became clear that apparent morphological differences between *Toxoplasma* and *Encephalitozoon* were insufficient to distinguish between them in sections and certainly could not serve as a basis for identifying an unknown parasite as one or the other. In retrospect the staining properties of the microorganisms might have furnished a minor clue to the identity of the protozoon of the human disease. It is stained easily with all the common stains and this is true of *Toxoplasma* of animal origin. *Encephalitozoon*, on the other hand, is stained by the carbol fuchsin stain and is less readily brought out by other common stains. Further, some features of the pathological lesions in the human disease, as noted above, are more like those

encountered in experimental toxoplasmosis in the rabbit than spontaneous Encephalitozoon infection in that animal. The granulomas in the central nervous system in Encephalitozoon infection often show central necrosis, while in this human disease and animal toxoplasmosis they do not. No diffuse inflammatory and necrotizing lesions are present in the brain in Encephalitozoon infection, whereas they are prominent in the human disease and occur in experimental toxoplasmosis. Spontaneous Encephalitozoon\* infection is chronic, and in the majority of instances non-lethal, while the human disease and experimental toxoplasmosis, as a rule, are subacute and fatal. In the 1 case of the human disease in which other organs were involved, the heart showed lesions and the kidneys did not. This is more like toxoplasmosis than Encephalitozoon infection. Some of these points were discussed in the report of new observations in Richter's case, and it was pointed out there that the possibility that the human infection was toxoplasmosis rather than Encephalitozoon infection would have to be seriously considered. It was obvious, however, that until the human infection could be transmitted to animals and the microorganism and the experimental infection produced by it studied and compared with toxoplasmosis, no decision could be made.

The transmission of the infection from the infant (present case) to animals and the experimental studies<sup>69</sup> which followed yielded definite evidence that the causative microorganism in this case is a *Toxoplasma*. (1) The morphology of the microorganism isolated from the human case corresponded to that of *Toxoplasma* of animal origin. (2) The course of the disease and the lesions produced in the animals inoculated with it were similar to those noted in the same species by inoculation of a *Toxoplasma* of animal origin. (3) The susceptibility of the rabbit, mouse, guinea pig and chick to this microorganism corresponded to the wide host range of *Toxoplasma* of animal origin. (4) Convincing evidence

\* The meager knowledge of Encephalitozoon is due to the fact that so few have been able to transmit the infection experimentally (and then with equivocal results<sup>77</sup>), and that it has not been possible to cultivate the microorganism. The resemblances between it and *Toxoplasma* make one wonder whether there may not be some close relationship between the two microorganisms. Our failure to transmit an Encephalitozoon infection has prevented us from directly comparing the biological characteristics of this parasite with those of *Toxoplasma*. It is remarkable that others who have worked with one or both of these organisms have not troubled to make any exact and clarifying comparison between them.

of the nature of the microorganism was obtained by cross-immunity experiments. Rabbits and mice immune to the human strain proved to be immune to the Rockefeller Institute guinea pig strain of *Toxoplasma* and *vice versa*. This experimental material will be described in detail in a forthcoming report. Although experimental evidence is lacking to establish the identity of the protozoa in the other 4 cases (our Case 1, and those of Jankû, Torres and Richter), their morphological resemblance to this proved *Toxoplasma* and the similarity of the lesions produced make it extremely probable that they, too, are *Toxoplasmata*. The disease represented by these 5 cases might, therefore, be designated toxoplasmic encephalomyelitis and the causative microorganism, *Toxoplasma hominis*.

Three cases of obscure human infections which have at times been cited as possible instances of toxoplasmosis do not withstand critical analysis. The 1st case was reported by Castellani<sup>10</sup> (1914) from Ceylon in a boy of 14 who had a prolonged fever, splenomegaly and severe anemia, and was markedly emaciated at death. The organs were described as normal grossly except for the spleen which was greatly enlarged. The nervous system was apparently not examined. No histological examination was reported. Smears of the blood and spleen revealed bodies that Castellani decided were *Toxoplasmata* and named *Toxoplasma pyrogenes*. There were two types, the larger of which measured 7-12 $\mu$  in maximum diameter. They were rounded or pear shaped and contained several large masses of chromatin. The smaller measured 2.5-6 $\mu$  in maximum diameter, were round, oval or crescentic in shape, and had one large rounded mass of chromatin at one pole or centrally placed. Wenyon<sup>65</sup> in discussing this case voiced a justifiable doubt as to the nature of the structures described. The larger forms he believed are portions of degenerated cells and the smaller forms probably vegetable organisms, like yeasts. The lack of any description of lesions in the organs which might be compared with those in animal toxoplasmosis renders any decision as to whether this was a toxoplasma infection or not even more difficult.

Fedorovitch<sup>16</sup> (1916) described a case of chronic fever in a boy of 10 years from the Black Sea district. This child also had anemia and a markedly enlarged spleen. Blood smears revealed

an organism which the author considered to be very much like the bodies described by Castellani. These were not present in material from a puncture of the spleen. The fate of the patient is not known and there is, therefore, no clue as to what pathological lesions may have been present. Wenyon<sup>65</sup> was of the opinion that the bodies were vegetable organisms like large cocci or yeasts.

Chalmers and Kamar<sup>11</sup> (1920) observed soldiers in the Sudan with chronic fever, headache, slight cough and diarrhea, and terminal severe anemia and gingivitis. The pathological lesions in an autopsy performed on 1 individual were not recorded except for a photograph of a splenic film. This illustrates bodies which the authors do not describe but state are similar to those Castellani considered to be *Toxoplasmata*. Wenyon<sup>65</sup> believed these bodies to have been altered *Leishmania*.

It is clearly impossible to determine whether or not these are cases of human toxoplasmosis. The pathological lesions are not described so that a comparison cannot be made with the lesions in animal toxoplasmosis, and transmission to animals was not attempted for the purpose of identifying the organisms. Although the morphology of some of the single bodies may suggest *Toxoplasma*, cysts are not described and some of the structures illustrated are quite unlike *Toxoplasma*.

That there may be forms of human toxoplasmosis other than granulomatous encephalomyelitis of infants is possible. One cannot, however, accept these 3 cases as instances of another type of toxoplasmosis in the face of the inadequacy of the data concerning them.

From a review of the 5 cases of toxoplasmic encephalomyelitis, the disease is found to have the following characteristics:

*Clinical Features:* Although the records are not always complete, the clinical data in this series of patients are sufficiently alike to furnish at least the outlines of a syndrome (Table I). The patients were all infants, 4 of whom died before the age of 2 months. The youngest of these was 2 days of age and the oldest 7 weeks, the duration of the illness having varied from 2 to 28 days. The age at death in the 5th case, that of Jankû, was not clear from the record, but appears to have been the 11th or 16th month, and the duration of the illness was not exactly stated, but

TABLE I

Summary of Clinical Data in Five Cases of *Toxoplasmic Encephalomyelitis*

Case	Location	Sex	Age at death	Duration of illness	Symptoms and signs	Ophthalmoscopic examination	Spinal fluid
Authors' 1st case J. S. <sup>37</sup>	New York City	F	30 days	28 days	Convulsions, diarrhea, vomiting, labile body temperature, slight enlargement of head (?), disturbances in respiration	Yellowish white focal area of chorioretinitis, bilateral	Xanthochromia, high protein content, pleocytosis (largely mononuclears), 2 eosinophils in smears
Janků <sup>21</sup>	Prague, Czechoslovakia	M	11-16 mos. (?)	8-11 mos. (?)	Marked hydrocephalus, blindness, convulsions, anorexia, vomiting	Yellowish white focal areas of chorioretinitis bordered by pigment in macular region bilaterally	
Torres <sup>50, 60, 61</sup>	Rio de Janeiro	F	2 days	2 days (?)	Convulsions		
Richter <sup>40</sup>	Chicago, Ill.	F	7 weeks	1 week (?)	Convulsions, opisthotonos, fever, "cold"		Xanthochromia, high protein content, pleocytosis (mild, cell type not stated)
Authors' 2nd case C. D.	New York City	M	31 days	28 days	Convulsions, vomiting, sensory and motor signs of cervical cord lesions with subarachnoid block, labile body temperature, disturbances in respiration	Reddish brown areas of chorioretinitis in macular regions bilaterally	Cloudy xanthochromic fluid, high protein content, clotted spontaneously (no microscopic examination)

seems to have been at least 8 months. Two of the infants were males and 3 were females.\* All suffered convulsions at some time or other during their illness. Two had enlargement of the head due to internal hydrocephalus. In 3 of the infants there was a labile body temperature with occasional mild fever or subnormal temperature. In 3, vomiting occurred at intervals. The spinal fluid from 3 of the patients was examined and showed xanthochromia and a high protein content, and in 2 there was pleocytosis. The spinal fluid smear in 1 case revealed occasional eosinophils in addition to mononuclear cells. In this case parasites were identified in paraffin sections of precipitated ventricular fluid. An ophthalmoscopic examination was made of 3 of the infants and in all a focal chorioretinal lesion was found in each eye. The disease, then, appears to affect young infants, produces manifestations of involvement of the nervous system, may give rise to ophthalmoscopically identifiable focal lesions in the eyegrounds, and terminates fatally after an acute or subacute course.

*Gross Pathology:* The pathological picture of the disease is distinctive. The central nervous system is the site of the most severe and widespread changes. Macroscopically depressed, softened, yellowish focal lesions varying in size from a few millimeters to 3 cm. in diameter are seen on the surface of the cerebrum. In these areas the convolutional markings are obliterated and the overlying leptomeninges are thickened and rendered opaque by a grayish yellow exudate. There may be swelling of the gyri at the margins of these lesions while the intervening gyri and their overlying leptomeninges appear normal, except for a varying amount of congestion. The spinal cord may show similar focal areas of softening and collapse, or considerable localized swelling with an accompanying leptomeningeal reaction limited to such regions.

The cerebral lesions are often confined to the cortex but may involve the subcortical white matter and centrum ovale and reach the wall of the lateral ventricle. They are well demarcated and range in size from minute nodules to patches several cm. in diameter. The center of such lesions is yellowish, soft, and sometimes cheesy, while the edges may be grayish and glassy. In some

\* Torres' patient, whose sex is not given in the author's report, was a female (personal communication).

of the cerebral foci gritty material may be palpable. Others may contain cysts which occasionally reach a diameter of several cm. Focal changes similar to those in the cortex and white matter are encountered in the corpus striatum, thalamus and hypothalamus, and may occur in the midbrain, pons and medulla as well. The cerebellum shows minor lesions in 1 case. There may be a varying degree of enlargement of the lateral and third ventricles due in part to stenosis of the aqueduct of Sylvius, and partly to degeneration of the ventricular walls. The latter may be a prominent gross lesion and is seen as a well demarcated broad band of yellowish, soft, friable periventricular necrotic tissue. It is associated with extensive denudation of the ependymal lining. Coarse ependymal granulations may be present in the better preserved portions of the ventricular walls. The lesions in the spinal cord may also vary from minute yellow foci to large confluent softenings involving the entire diameter of the cord in a number of segments, obliterating all the normal architectural markings, and leaving only yellow pultaceous material.

A complete autopsy was performed in 3 of the 5 cases: Torres', Richter's, and our 1st case. The last 2 cases showed no pertinent gross changes in any of the viscera, bones or muscles. Torres gave no gross description of his material except for a brief reference to the brain, although microscopic changes were referred to in some of the other organs, as will be noted below.

*Microscopic Examination:* The histological changes are characteristic. There is a widely disseminated encephalomyelitis in which the severest lesions are associated with necrosis. There is a total destruction of neural and neuroglial elements in the areas of degeneration leaving amorphous debris. In the marginal necrotic material, lipoid laden phagocytes and often polymorphonuclear leukocytes are present. There is a bordering zone of reaction containing hyperplastic capillaries, plasma cells, lymphocytes, mononuclear leukocytes, neutrophils and eosinophils. Fibroblasts derived from hyperplastic capillaries and from the contiguous leptomeninges often mingle with these cells, and in places where the capillary proliferation is intense, give rise to a rich granulation tissue in which many collagen and reticulum fibers may occur. Beyond the zone of cellular infiltration there is a moderate astrogliosis as well as hypertrophy and multiplication of microglial cells



with production of rod cells and transitional phagocyte forms. Cavities may occur in the necrotic zones and contain persisting hyperplastic capillaries and varying numbers of lipoid laden phagocytes. Degenerated tissue may become partially or completely calcified and give rise to broad bands of calcific material in the cortex or periventricular zone. Incrustation of nerve cells and other elements occurs in these areas.

Miliary granulomas constitute a prominent feature of the pathological process in the nervous system. They are found scattered through the brain and spinal cord and often cluster near the focal necrotic and inflammatory lesions. Each granuloma is composed of a circumscribed group of closely approximated epithelioid cells, evidently of capillary endothelial origin. Mingled with these, usually in the outer portion of the granuloma, are varying numbers of plasma cells, lymphocytes, mononuclear leukocytes and eosinophils.

The leptomeninges overlying the parenchymal lesions show a moderate hyperplasia of their cells with the production of fibroblasts, and they are infiltrated by cells similar to those seen in the parenchymal exudate. Here again plasma cells and eosinophils are a characteristic feature of the inflammation. Often the exudate is continuous from pia-arachnoid to parenchyma, obliterating the line of demarcation between them. Many of the leptomeningeal capillaries in these areas of inflammation and some of the larger vessels show endothelial hyperplasia. There is no occlusion of leptomeningeal or parenchymal vessels to account for the areas of necrosis, although secondary thrombosis is encountered. Infiltration of the connective tissue core of the choroid tufts and tela choroidea may be present. The walls of the lateral and third ventricles are frequently denuded of ependyma and may be covered by exudate. Where the ependyma is intact there are at times numerous ependymal granulations and subependymal gliosis. This may lead to stenosis of the aqueduct of Sylvius.

The focal lesions in the eyes consist of patches of severe chorioretinitis. The involved retina is swollen and shows varying degrees of degeneration. Total necrosis may occur in some areas and involve many of the layers. The internal limiting membrane is at times disrupted. At the margins of completely necrotic areas the cells of the ganglionic layer are degenerated or disappear.

The deeper layers, although edematous, are better preserved except for the layer of rods and cones and the pigmented layer, which are often necrotic and disrupted. Free pigment granules as well as dislocated pigmented cells are often extruded into the internal retinal layers. There may be scattered plasma cells and lymphocytes in the necrotic areas with moderate infiltration by similar cells as well as occasional eosinophils, neutrophils, mononuclear leukocytes and lipoid laden phagocytes in the marginal zone. In the more severely affected layers of the retina there is an increase in glial tissue and formation of granulation tissue. The latter may invade the posterior chamber. An exudate similar to that described above may be present on the internal surface of the retina. The choroid is congested, hyperplastic, and infiltrated chiefly by plasma cells and lymphocytes. A mild similar infiltration may occur in the sheath of the optic nerve. The sclera remains unchanged.

Inflammatory lesions may occur in other organs, as reported in Torres' case. This author mentions the presence of "disseminated foci of myositis" in the striated muscles, and "acute diffuse myocarditis" in which the predominant cells are mononuclear in type with an admixture of numerous eosinophils. Complete autopsies in 2 other cases revealed no lesions in the other organs except for a terminal bronchopneumonia in 1.

*Microorganisms:* A comparison of the morphology of the parasites in the 5 cases referred to in this paper makes it seem probable that we are dealing with the same organism, or at least closely related forms in each instance. The parasites seen in sections of fixed tissue, using any of the common stains, are usually ovoid corpuscles with a spherical polar chromatin body. They may be oval, pyriform, rounded or occasionally lunate, and the chromatin body may rarely be centrally placed and band-like. They also occur less frequently in clusters which have been described as cysts. The cysts are composed of varying numbers of closely packed organisms which for the most part are clearly outlined but sometimes seem to form a syncytial mass. A cyst wall appears to be present but this may be the margin of a matrix in which the parasites are embedded. The apparent cyst wall, on the other hand, may be the remains of the cytoplasm of a parasitized cell.

Parasites are present in small numbers in the leptomeningeal exudate. Large numbers are found in the inflammatory tissue at the margins of the necrotic parenchymal lesions, and numerous degenerated forms are present in the central débris. Moderate numbers of parasites occur in the granulomas. They are abundant in the focal chorioretinal lesions. In the 1 case (Torres) in which other organs were involved, namely the heart and striated muscles, parasites were seen in the lesions and microorganisms were also described in the subcutaneous tissue.

The parasites may be free or intracellular. The intracellular forms occur in large mononuclear cells and epithelioid cells of granulomas, less frequently in polymorphonuclear leukocytes, and rarely in the endothelial cells of the capillary walls, eosinophils or nerve cells. It is probable that they occur in other cells such as ependymal, choroidal and leptomeningeal elements, but as yet they have not been found parasitizing such cells.

Some minor differences in the size and morphology of the parasites in the 5 cases do not appear to be significant. The majority of the single organisms as described above in paraffin sections of formalin and Zenker-fixed tissue measure  $2-3\mu$  in length and  $1.5-2\mu$  in width. Torres finds the length of the individual organisms to be slightly greater ( $3.5\mu$ ), some of those in the muscles reaching a length of  $6\mu$ . In Richter's material the parasites measured  $2-2.5\mu$  in length and  $1.5-2\mu$  in width, figures slightly smaller than those given in our cases. All of these measurements are average figures and do not indicate the overlapping which occurs when the extremes are included. In any case the differences in fixation and in preparation of the tissues may well account for the slight dissimilarities noted. The variation in the size of the cysts, particularly the larger average sizes given by Jankû, probably depend on similar factors and do not appear to be important. The apparent differences between the appearance of the microorganisms in these human cases and that of *Toxoplasma*, as generally described in the literature, would seem to depend on differences in histological technic. As has been demonstrated, *Toxoplasma* from an animal source, when seen in sections of embedded tissue, are exactly like those in the human cases, while the microorganisms in smears assume the larger lunate form commonly described for *Toxoplasma*.

*Mode and Source of Infection:* In considering the mode of infection in 3 of these cases, in a previous paper it was pointed out that the onset of the clinical manifestations of the disease soon after birth in 2 cases, and the advanced nature of the lesions, suggested that the infection had begun during intrauterine life. This is supported by the evidence derived from the case reported here in which symptoms began on the 3rd day of life. Although blindness was first noted in Jankû's patient at 3 months of age, Jankû considered the infection congenital since a maldevelopment of one eye was present which he thought ascribable to infection during fetal life. In Richter's case the infant was said to have become ill at the age of 6 weeks, but the incompleteness of the clinical data permits a doubt as to whether the onset was not at an earlier age. It was Richter's opinion that the infection was congenital in view of the apparently advanced age of some of the pathological changes. As in the case described here, there was marked calcification of many of the focal lesions.

That the mothers of the 5 infants were apparently in good health does not preclude the possibility of the infection having occurred *in utero*. It is conceivable that they were carriers of a clinically inapparent *Toxoplasma* infection to which the fetus was more susceptible. *Toxoplasma* have often been found in animals which showed no clinical symptoms, although the virulence of the parasites has been demonstrated by their ability to produce active infection when inoculated into other animals. If the infection was transmitted *in utero*, it might be expected that the placenta would show specific pathological changes. Unfortunately the placentas were not examined in any of these cases. It is to be hoped that in the future such an examination will be made. How the mothers might have been infected, or the children, if they acquired the infection independently after birth, is not clear.

As mentioned above, *Toxoplasma* has a widespread geographic distribution, and pathogenicity for a wide variety of hosts, but its natural mode of transmission is as yet unknown. Experimentally toxoplasmosis has been successfully transmitted by inoculation of infected material by many routes.<sup>23</sup> Infection has followed cannibalization<sup>51</sup> of animals recently dead of the disease. Instillation of infected peritoneal fluid into various cavities lined by mucous

membrane, including the vagina and conjunctival sac, has resulted in a generalized infection in some instances.<sup>36</sup>

As has been noted previously, Jankû, apparently unaware of the occurrence of spontaneous *Toxoplasma* infection in rabbits, alluded to the fact that the mother of the infant he described had been resident during her pregnancy on a farm where rabbits were raised, and that she had eaten much rabbit meat during the period of gestation. In Torres' and Richter's reports no reference was made to contact of the mother with animals. It is of interest in relation to Torres' case that Brazilian rabbits and birds are known to be infected with *Toxoplasma*. In our 1st case the home was so overrun with mice that the mother changed her residence in the midst of her pregnancy. Although spontaneous toxoplasmosis has not been described in mice in North America, its occurrence in that species is known in other parts of the world. There was also a canary in the home during the pregnancy in our 1st case. These birds have been found to be subject to toxoplasma infection. In the case reported here there was no history of contact with animals.

In the 2 instances in which there was contact of the mother with animal species which may harbor *Toxoplasma* there is a possibility that transmission occurred by ingestion of infected material, *i.e.*, rabbit meat in Jankû's case, and food contaminated by mouse excreta in our 1st case. The existence of an insect or other vector as an intermediate host between a possible animal carrier and man cannot be excluded. The portal of entry of *Toxoplasma* in the mother and its localization prior to infection of the infant remain undetermined in the light of our present knowledge. In view of the report of experimental vaginal infection with *Toxoplasma* one must consider the possibility of vaginal infection in the mothers with direct extension of the infection to the uterine contents. This hypothesis seems unlikely.

The possibility that a maternal vaginal infection was transmitted to the infant in its passage through the birth canal is excluded in our 2nd case in view of the fact that delivery was by Cesarean section, and by analogy is unlikely in the other instances.

If the infection be congenital it is probable that it occurs late in fetal life since developmental anomalies were absent except in 1 instance (malformation of the eye in Jankû's case). It is clear that

no absolute evidence exists that the infants became infected before birth. This problem has yet to be attacked experimentally in animals and evidence sought in future human cases.

*Pathogenesis:* The description of the probable mode of spread of the infection, the susceptibility of various organs and tissues, and the development of the lesions, as summarized in the discussion of our 1st case, apply to the case reported here as well. No new facts as to the pathogenesis of the disease have been added by a study of the pathological material from the present case. Study of the characteristics of the infection in animals has afforded an opportunity to test these hypotheses of the pathogenesis of the human disease and they will be discussed in the presentation of the experimental material.

*Diagnosis:* Although it may be premature to attempt to establish criteria for the clinical diagnosis of toxoplasmic encephalomyelitis, it is useful to summarize the facts available to date which may lead to a recognition of the disease during life. Young infants of either sex become ill soon after birth. They are subject to repeated convulsions and may show symptoms and signs of widespread involvement of the central nervous system. Ophthalmoscopically, focal areas of chorioretinitis are seen in each eye. These appear as yellowish white or brownish red, round or oval patches which may show irregular black, often marginal pigmentation. The intense calcification of the cerebral lesions in some of these cases suggests that stereoroentgenograms of the skull may reveal the presence of such changes. The cerebrospinal fluid is xanthochromic, contains a large amount of protein, and shows a pleocytosis chiefly of round cells. Eosinophils have been found in ventricular fluid. Toxoplasma have been identified in fluid from the same source and it seems probable that intracerebral inoculation of such fluid into rabbits or mice will demonstrate the presence of the parasite by the production of a specific infection.

#### SUMMARY AND CONCLUSIONS

1. A 5th case of a new disease, granulomatous encephalomyelitis due to a protozoon, occurring in an infant is described.

2. The clinical and pathological observations in this case are shown to be similar to those in the first 4 cases. This group forms a distinct disease entity. The disease affects young infants, pro-

duces manifestations of generalized involvement of the nervous system, may give rise to ophthalmoscopically identifiable focal lesions in the eyegrounds, and terminates fatally after an acute or subacute course. The spinal fluid shows xanthochromia, a high protein content and pleocytosis. The central nervous system is the site of focal inflammatory and degenerative lesions which are widely disseminated. Similar changes are found in the retina and choroid. Miliary granulomas are a characteristic feature of the process in the nervous system. Focal inflammatory lesions were present in the heart and striated muscle in 1 case.

3. A protozoan parasite is present in all the lesions.

4. The results of transmission of the infection to animals from the case reported here indicate that the causative protozoon is a *Toxoplasma*. The designation *Toxoplasma hominis* is suggested for the microorganism and the term toxoplasmic encephalomyelitis for the disease.

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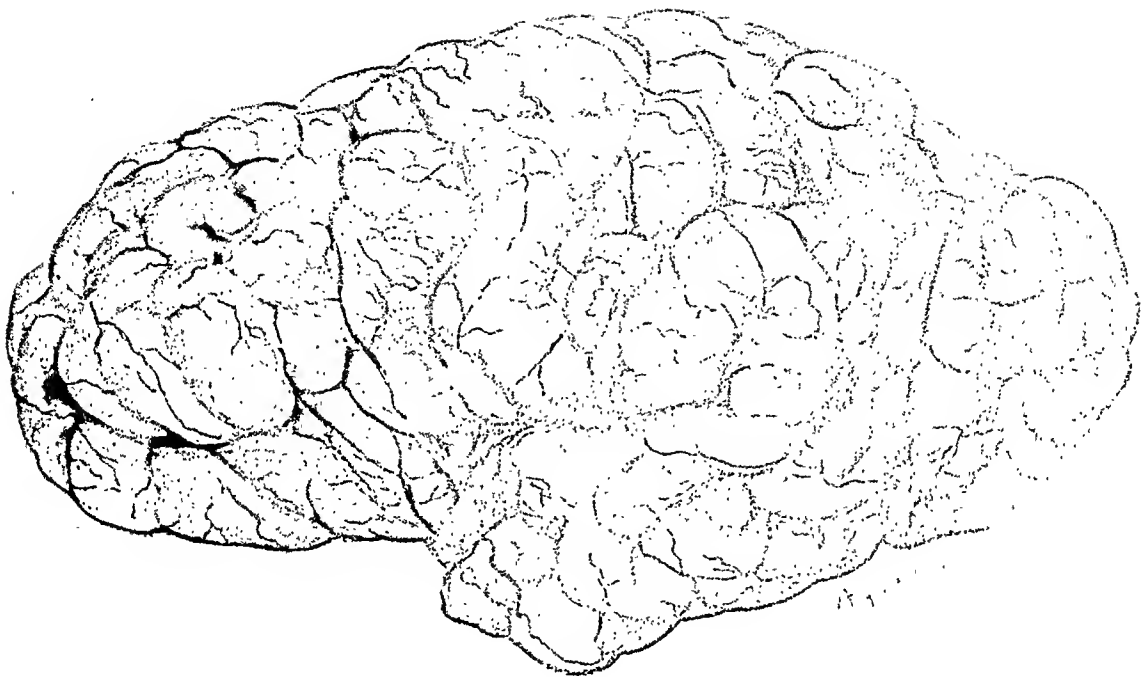
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## DESCRIPTION OF PLATES

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### PLATE 99

FIG. 1. Left cerebral hemisphere showing depressed, yellowish and softened cortical lesions.



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Wolf, Cowen and Paige

Toxoplasmic Encephalomyelitis

PLATE 100

- FIG. 2. Left cerebral hemisphere, coronal section. Focal necrotizing lesions are present in the cortex and in the subcortical white matter.
- FIG. 3. Cervical spinal cord showing swelling of cord and obliteration of normal architectural markings.
- FIG. 4. Upper thoracic spinal cord showing discoloration with obscuration of normal markings.
- FIG. 5. Lower thoracic spinal cord showing changes similar to those present in Fig. 4.
- FIG. 6. Lumbar spinal cord showing obliteration of posterior columns and horns by grayish tissue.
- FIG. 7. Pons. A sharply demarcated focal lesion is present at the junction of the tegmentum and the reticular zone.

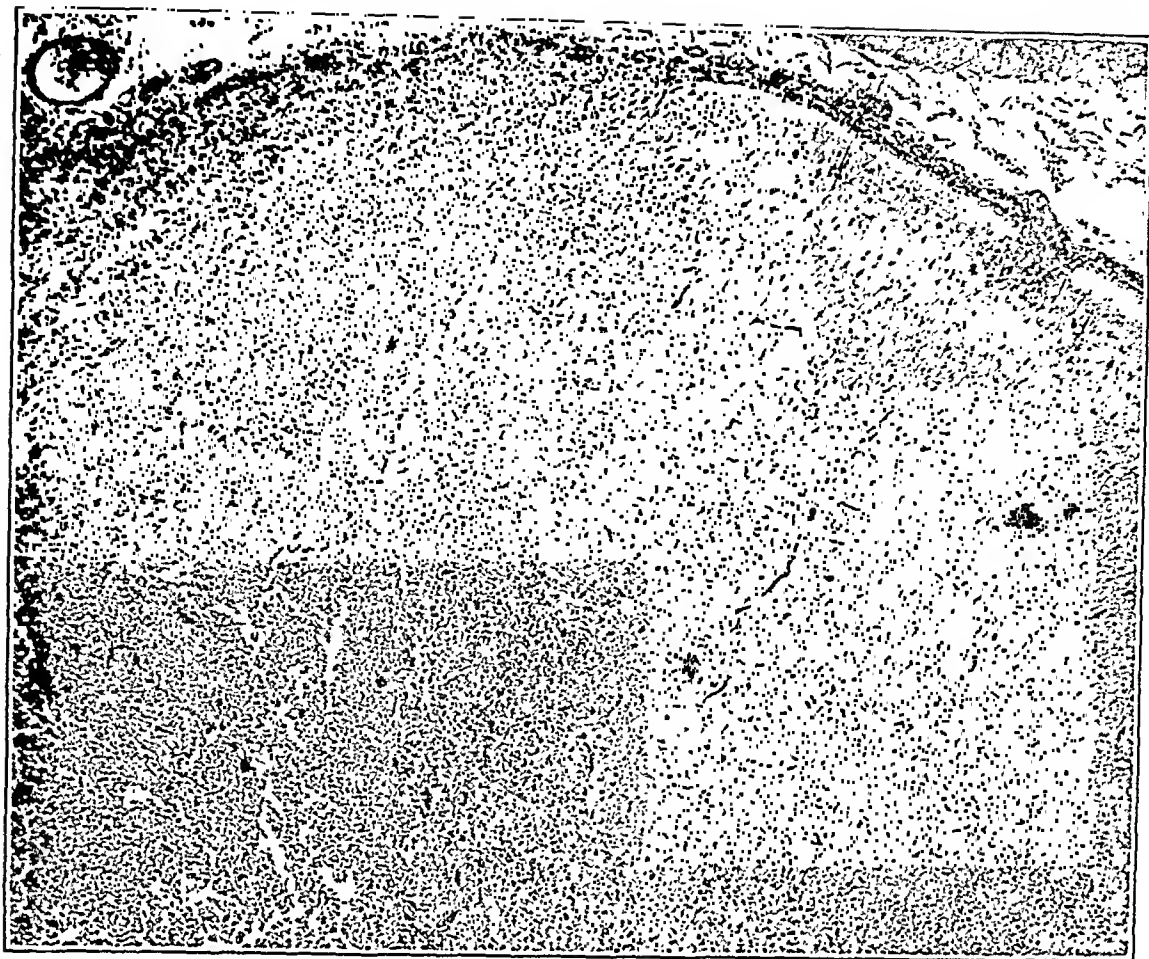


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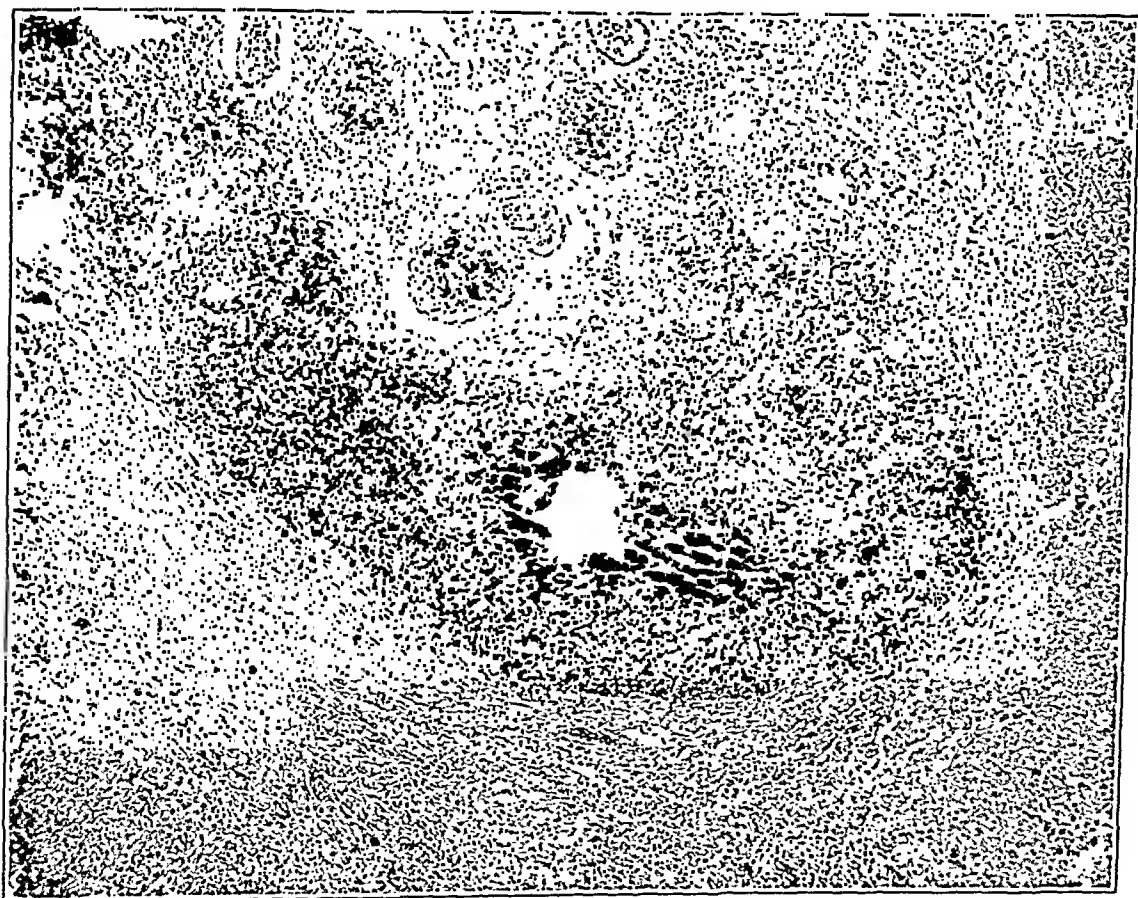
Toxoplasmic Encephalomyelitis

PLATE 101

- FIG. 8. Cerebral cortex. A focal inflammatory and necrotizing lesion is present in the cortex. Note the comparatively sharp margin and accompanying focal leptomeningitis. The adjacent cortex on the right is relatively well preserved. Hematoxylin-eosin stain.  $\times 80$ .
- FIG. 9. Cerebral cortex. Note the marked calcification of a necrotizing cortical lesion. Hematoxylin-eosin stain.  $\times 80$ .



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PLATE 102

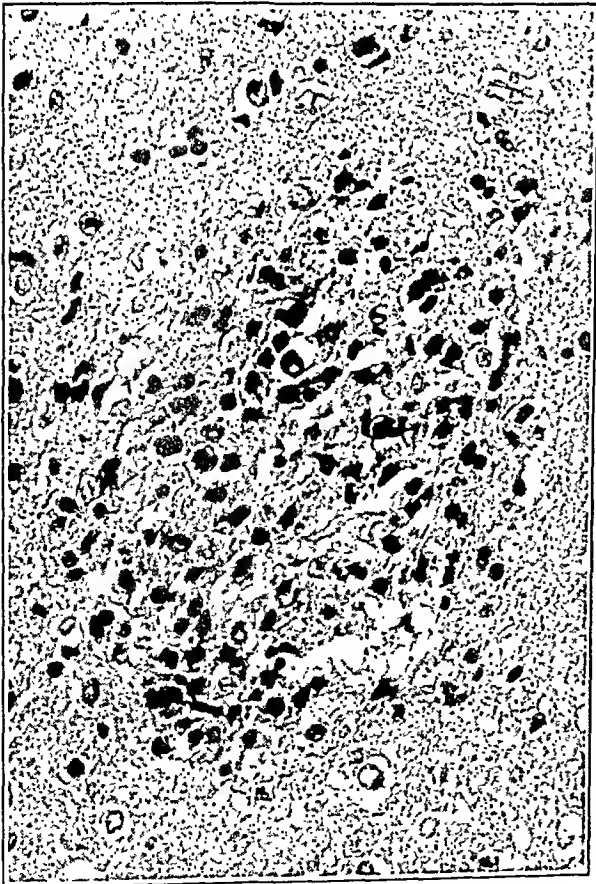
- FIG. 10. Cerebral cortex. A granuloma is present in the third cortical layer adjacent to a blood vessel. Focal leptomeningitis. Hematoxylin-eosin stain.  $\times 150$ .
- FIG. 11. Cerebral leptomeninges. Focal leptomeningitis. Lymphocytes, plasma cells and large mononuclear leukocytes are present in the exudate. Lipoid laden phagocytes, lymphocytes and plasma cells are seen in the underlying degenerated cortex. Hematoxylin-eosin stain.  $\times 300$ .
- FIG. 12. Lumbar spinal cord. White matter showing a granuloma composed of epithelioid cells and a few lymphocytes. Hematoxylin-eosin stain.  $\times 300$ .
- FIG. 13. Wall of the lateral ventricle. Inflammation, necrosis and loss of the ependymal lining are present. Hematoxylin-eosin stain.  $\times 150$ .



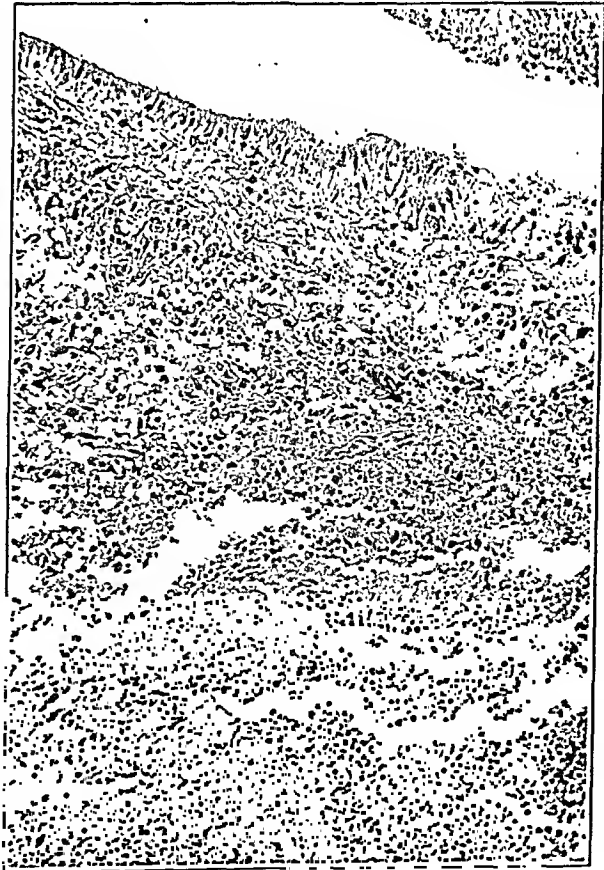
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PLATE 103

- FIG. 14. Cervical cord. Section of anterior horn showing an acute inflammatory and necrotizing lesion with infiltration by polymorphonuclear leukocytes, lymphocytes and large mononuclear cells. Hematoxylin-eosin stain.  $\times 250$ .
- FIG. 15. Cerebral cortex. Calcification is seen in a degenerated focal lesion in the cortex. Note the encrusted nerve cells. Hematoxylin-eosin stain.  $\times 200$ .
- FIG. 16. Granuloma with surrounding astrocytosis. The epithelioid cells in the granuloma are obviously unrelated to astrocytes. Cajal's gold sublimate stain.  $\times 300$ .
- FIG. 17. Lumbar spinal cord. Miliary granulomas in the lateral and the anterior white columns. Giemsa's stain.  $\times 150$ .



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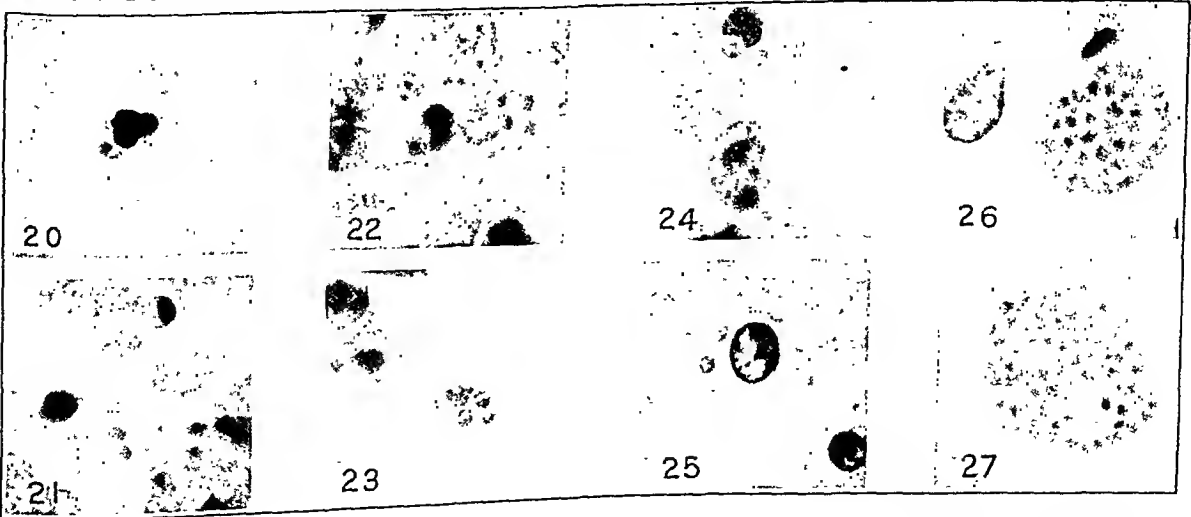
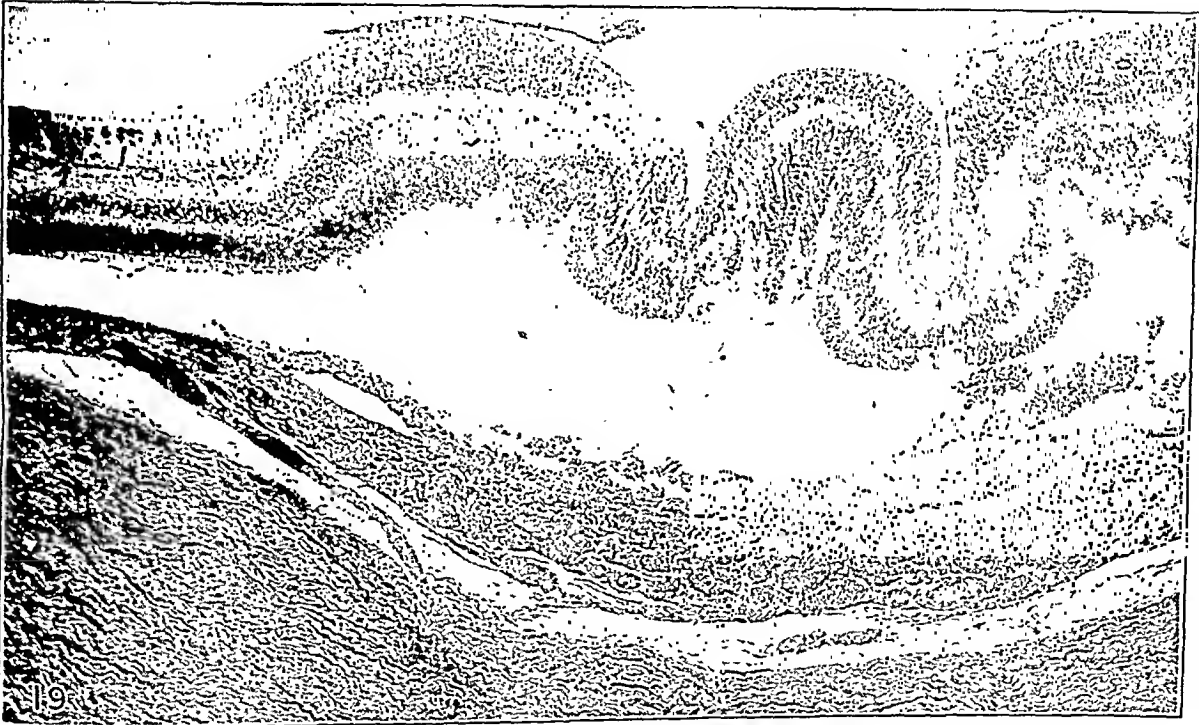
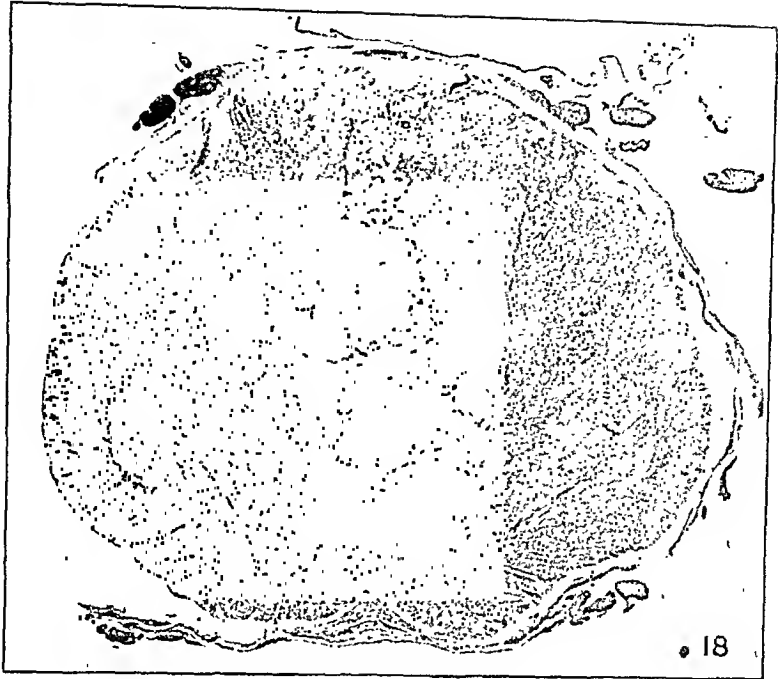


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Toxoplasmic Encephalomyelitis

PLATE 104

- FIG. 18. Lumbar cord. Note the intense necrosis of the posterior and portions of the lateral columns, the posterior and parts of the anterior horns and the central gray matter and commissures. Pal-Weigert stain.  $\times 20$ .
- FIG. 19. Retina of the right eye. Note the edema and degeneration of the retina and infiltration of the choroid. Hematoxylin-eosin stain.  $\times 40$ .
- FIGS. 20-27. Show parasites in lesions in the cervical cord and pons.  $\times 1050$ .
- FIG. 20. Single oval intracellular parasite with polar chromatin body. Phloxine-hematoxylin stain.
- FIG. 21. Two free ovoid parasites. Hematoxylin-eosin stain.
- FIG. 22. Four free oval parasites. Hematoxylin-eosin stain.
- FIG. 23. Three intracellular round and oval parasites. Hematoxylin-eosin stain.
- FIG. 24. One round intracellular parasite showing division of its chromatin body. Hematoxylin-eosin stain.
- FIG. 25. Two intracellular parasites, one binucleated, apparently dividing. Hematoxylin-eosin stain.
- FIG. 26. Parasitic cyst. Hematoxylin-eosin stain.
- FIG. 27. Parasitic cyst rupturing and liberating parasites. Hematoxylin-eosin stain.





## EXPERIMENTAL PRODUCTION OF ENDOCARDITIS LENTA \*

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Rosenbach<sup>1</sup> in 1878 passed an instrument down the right carotid artery in dogs and rabbits to wound the valves of the heart. Two of his dogs, apparently through unintentional infection, developed large vegetations on the aortic valves with septic infarcts and petechial hemorrhages in various organs. Colonies of organisms, apparently staphylococci, were demonstrated in these lesions. Ribbert<sup>2</sup> later succeeded in producing endocardial lesions without direct wounding of the valves by injecting into the ear vein of the rabbit a suspension of staphylococci from potato cultures so prepared as to include starch grains and larger fragments of potato. Large doses were required and in the successful experiments the animals died within 60 hours. Ribbert recognized that the disease was unlike human endocarditis. Dietrich<sup>3</sup> has more recently elaborated the experiments of Ribbert by introducing various substances parenterally to injure the valves of the heart and then injecting staphylococci or colon bacilli to cause the bacterial vegetations.

Dreschfeld<sup>4</sup> in 1887 appears to have been the first to transmit to experimental animals streptococci from a case of fatal human endocarditis, with resultant vegetations on the heart valves, which he accomplished by merely injecting the culture into the jugular vein of rabbits. It now seems probable that this investigator actually transmitted to rabbits the specific variety of endocarditis associated with the presence of *Streptococcus viridans*, although this organism was not clearly distinguished from other streptococci in 1887. Some 20 years later, Horder<sup>5</sup> injected 10 cc. of a broth culture of *Streptococcus salivarius* (*viridans*), isolated from the blood of a patient with chronic malignant endocarditis, intravenously into a rabbit weighing 2170 gm. The injection was repeated 10 days later. After a further interval of 38 days the rabbit died

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and at autopsy there were massive mitral vegetations projecting into the auricle and containing the same streptococcus. Similar results were obtained with streptococci isolated from normal human saliva and normal human feces. Photographs of the lesions are shown in his publication. Rosenow<sup>6</sup> in 1912 used streptococci of the viridans group isolated from the blood and from the tonsils of endocarditis patients and injected single large doses or repeated smaller doses intravenously into rabbits. Many of his animals succumbed in a few days but some of them survived for longer periods and in these he observed gross vegetations on the heart valves and in some the scars of healed valve lesions. Single very large doses or repeated smaller doses given intravenously to half grown rabbits proved to be the most successful procedure. Old rabbits were resistant and in young rabbits the lesions tended to heal quickly. Later Rosenow<sup>7</sup> used eight strains of *Streptococcus viridans* from cases of chronic septic endocarditis and produced lesions in 84 per cent of 44 rabbits injected. Apparently these animals were killed a day or two after inoculation and the small lesions were of somewhat uncertain significance for the endocarditis problem.

Experimental endocarditis produced by bacteria other than the *Streptococcus viridans* from human cases of endocarditis has been reported many times. Particularly significant is the report of Lloyd-Jones<sup>8</sup> who induced endocarditis in rabbits with great regularity by repeated daily intravenous injections of hemolytic streptococci, and particularly by such repeated injection of serum-broth cultures of viridans types of streptococci isolated from the human intestine and uterine cervix. More recently Bland and her associates<sup>9</sup> injected a particular strain of beta-hemolytic streptococcus intravenously into dogs with production of endocardial vegetations in 40 per cent of the animals. There are also numerous reports of experimental endocarditis produced by mechanical wounding of the endocardium or by the introduction of gross foreign bodies in addition to the bacterial inoculation, recalling the pioneer observations of Rosenbach. Thus Welch and his associates<sup>10</sup> wounded the endocardium with a needle and resorted to accessory respiratory infection and dietary restriction in order to lower the resistance of their experimental animals. Friedman and his associates<sup>11</sup> anchored within the heart or great vessels a minute

Bakelite capsule enclosing a culture of the bacteria. Kinsella and Muether<sup>12</sup> passed a metal rod down the right carotid artery to wound the valves and subsequently introduced the bacteria by intravenous injection and by feeding. Such technical procedures are essentially different from the methods of Dreschfeld and of Horder.

Some of our earlier, less conclusive experiments may be briefly mentioned. For example, Rabbit 77 was injected intravenously with rather small doses of *Streptococcus viridans*, strain D, a suspension of approximately 5,000,000 bacterial cells daily from August 10 to Sept. 28, 1936; then 10,000,000 daily from September 29 to October 4; 15,000,000 daily from October 5 to 11, and 20,000,000 daily from October 12 to 29. Blood cultures taken from time to time just preceding the respective daily injection all remained sterile. The animal was sacrificed on Nov. 6, 1936, and the heart revealed no recognizable lesions. In 1937 we obtained our first positive result by injecting intravenously larger doses of living culture according to the technic of Lloyd-Jones. Rabbit 12 was inoculated with a culture of endocarditis strain M, 500,000,000 bacteria on Jan. 19, 1937, and increasingly larger doses on succeeding days up to 2.5 cc. of serum-broth culture on January 22, 23, 25 and 26, and 2 cc. on January 27. Blood cultures taken on January 23, 26 and 27 gave positive growth and the inoculations were discontinued. However, the blood cultures taken on January 28, 30 and February 2 remained negative. So after 12 days without inoculation the intravenous injections of culture were resumed, 2 cc. daily on February 9, 10, 11 and 12, and 1 cc. on February 13. The blood culture taken on February 12 became positive and hence the inoculations were again discontinued. Additional blood cultures taken on February 15 and 23 also gave a positive growth of the streptococcus. Without further treatment the rabbit died on March 4, 1937, and at autopsy there was found a gross vegetation on the mitral valve. Four other rabbits, 11, 19, 466 and 467, were inoculated by a similar technic with endocarditis streptococci, strains S, M, W and G, from different patients during the early months of 1937 without production of any convincing gross lesions on the heart valves. The experimental work was then interrupted.

During 1938 further experiments of this kind were undertaken

with strains of *Streptococcus viridans* obtained by blood culture from clinically typical endocarditis lenta. For our more successful inoculations the bacteria were grown in broth enriched with blood plasma or blood serum of the rabbit and the inoculations were made by injection into the ear vein of large doses of this whole culture, 1 to 6 cc., repeated daily for 6 days. After a pause of 48 hours, usually over Sunday, a blood culture was taken and then the series of 6 daily doses repeated. This program \* was continued for variable periods until the evidence of successive positive blood cultures and progressive loss of weight made us believe that endocardial vegetations were established. The inoculations were then discontinued and the disease allowed to take its natural course or, in some instances, various therapeutic procedures were undertaken. During the subsequent course of the disease observations of the body weight and frequent blood cultures were made. Autopsy was performed as soon as possible on all animals that died. A preliminary note in regard to 21 of these animals was presented at the March meeting of the Society for Experimental Biology and Medicine<sup>13</sup> and some of the specimens from the animals were shown in the exhibit of the International Museums Association at Richmond, Virginia, April 5, 1939.

For many of these more recent experiments we used endocarditis strain P, *Streptococcus viridans*, obtained repeatedly in pure culture from the blood of a patient with endocarditis lenta. The organism ferments dextrose, saccharose, maltose and inulin, but not lactose, salicin, mannite or raffinose. It is not very toxic. We have been able to inject intravenously into volunteer donors as much as 1,500,000,000 heat-killed bacteria of this strain without recognizable reaction. Ten rabbits were inoculated with this bacterial strain, beginning on Dec. 5, 1938. Nine of these died and were examined sufficiently for present use. The 10th animal has, as yet, been inadequately studied.

Rabbit 458, weighing 1100 gm., received intravenous injections of 1 cc. of an 18 hour serum-broth culture of this strain P on December 5, 6, 7, 8, 9 and 10, 1938. It died on December 11,

\* While this method has resulted in the production of gross endocardial vegetations with all three endocarditis strains employed, it now appears that the disease may sometimes be transmitted by very few injections and there is an indication that a bacterial strain may become more adapted to the rabbit by successive animal passages.

6 days after the first inoculation. At autopsy there was some reddish material adherent to the mitral valve, which was considered as a possible vegetation. Microscopic study of sections through this revealed a laminated clot adherent to the ventricular surface of the leaflet and to the mural endocardium at its base. The deeper part of this deposit contained many leukocytes and on top of this was a layer of red clot. No bacterial colonies were recognized in it. An early lesion of endocarditis was considered possible but not proved. We have not included such animals among our positive results. Rabbits 143, 148, 345 and 472 of this group died in the period December 16 to 27, or 11 to 22 days after inoculations were begun, and at autopsy their hearts appeared negative.

The other 4 rabbits, 469, 304, 302 and 465, died in the period December 23 to January 5, that is, 18 to 31 days after inoculations were begun, and all of these showed convincing gross vegetations on the cardiac valves. These animals were used for testing various therapeutic agents. The protocol of one of them will suffice for our present purpose. Rabbit 469, weighing 1390 gm., was injected intravenously with a serum-broth culture of strain P, 1 cc. daily December 5 to 10 and December 12 to 15, and 1.5 cc. daily December 16, 17 and 19, and 2 cc. daily December 20, 21 and 22, 1938. On December 23 the animal was given 1 cc. of culture intravenously and then by stomach tube 10 grains of sulcamfamide dissolved in 5 cc. of water. Death occurred 15 minutes later. At autopsy the posterior aortic cusp presented a remarkable picture, being thickened to form a mass 5 by 4 by 3 mm., with a perforation of the leaflet at the base of this mass. The right cusp also presented a marginal thickening 1 mm. in diameter. Otherwise the heart appeared negative. In the cortex of the left kidney were numerous hemorrhagic spots and on the surface of the spleen a single yellow elevation 1 by 2 mm. in area. Microscopic sections of the aortic vegetations revealed abundant colonies of streptococci in the fibrin and adjacent necrotic tissue, and within the viable fibroblastic tissue of the valve was a microscopic abscess with a large bacterial colony at its center. This viable tissue was richly infiltrated with leukocytes.

This same strain P was used to inoculate 12 rabbits of a second series, beginning on December 28, with daily intravenous injec-

tions of 4 cc. Some of these were used for experimental therapy. Up to Feb. 12, 1939, 8 of them had died and all of these had recognizable gross vegetations on the endocardium. These rabbits are listed in Table I and photographs of the hearts of Rabbits 303, 307 and 313 are shown in Plate 105. The protocol of one of them will serve to indicate the program of inoculation. Rabbit 316, weighing 1800 gm., received intravenous injections of serum-broth culture of strain P, 4 cc. daily on December 28, 29, 30 and 31 (1938) and January 1, 3, 4, 5, 6, 7, 9, 10 and 11 (1939), a total amount of 52 cc. in 13 injections. The animal died about 1 hour after the last injection. Immediate autopsy revealed 100 cc. of clear watery fluid in the peritoneal cavity, an enlarged spleen, 50 by 14 by 8 mm., a congested liver with lesions of coccidiosis, and about 50 cc. of clear fluid in each pleural cavity. Both lungs were marked by numerous subpleural hemorrhagic spots. The heart was enlarged and both auricles and the right ventricle were enormously dilated. Large vegetations, attaining a thickness of 5 mm., were found obstructing the mitral orifice. Microscopic sections confirmed the gross observations and revealed abundant colonies of streptococci in the mitral vegetations.

A third series of 12 rabbits was started on January 17. All of these were inoculated with cultures of the same strain P. Several of them were used for therapeutic tests. Nine of them had died by March 5, 1939, and the lesions noted at autopsy are indicated in Table I. Six of the 9 had gross endocardial vegetations. The hearts of Rabbits 318, 326, 329, 325 and 354 are shown in Plate 105. The protocol of 1 animal will serve to illustrate the group. Rabbit 325, weighing 2070 gm., received intravenous injections of serum-broth culture of strain P, 4 cc. daily (except Sunday) from January 17 to February 25 inclusive, a total of 116 cc. of the culture. Blood cultures taken on January 23, February 13, 20 and 23 were positive; those taken on January 31 and February 6 remained negative. The weight had fallen to 1870 gm. on February 27. On this day a dose of 250 mg. of sulfapyridine in 5 cc. of 5 per cent gum acacia was given by stomach tube. This dose was repeated twice on February 28 and once on March 1. Death occurred March 1 at 2.30 P.M. At autopsy there were a few small coccidial lesions in the liver. The heart was dilated and there were large mitral vegetations, one of these measuring 4 by 4 by 6 mm.

TABLE I

*Data on Experimental Rabbits Inoculated with Endocarditis Streptococci*

Rabbit No.	Strain	Initial inoculation	Death	Significant lesions
12	M	Jan. 19, 1937	Mar. 4, 1937	Mitral vegetation
11	S	Jan. 19, 1937	Mar. 12, 1937	Negative
19	M	Jan. 30, 1937	Apr. 12, 1937	Negative
466	W	Feb. 8, 1937	Feb. 11, 1937	Negative
467	G	Feb. 8, 1937	Feb. 15, 1937	Negative
458	P	Dec. 5, 1938	Dec. 11, 1938	Uncertain
143	P	Dec. 5, 1938	Dec. 16, 1938	Negative
148	P	Dec. 5, 1938	Dec. 19, 1938	Negative (?)
345	P	Dec. 5, 1938	Dec. 20, 1938	Negative (?)
469	P	Dec. 5, 1938	Dec. 23, 1938	Aortic vegetation, spleen
472	P	Dec. 5, 1938	Dec. 27, 1938	Negative
304	P	Dec. 5, 1938	Dec. 30, 1938	Mitral vegetations
302	P	Dec. 5, 1938	Dec. 30, 1938	Tricuspid vegetations, kidney
465	P	Dec. 5, 1938	Jan. 5, 1939	Mitral vegetation
316	P	Dec. 28, 1938	Jan. 11, 1939	Mitral, mural vegetations, kidney, lung
314	P	Dec. 28, 1938	Jan. 15, 1939	Tricuspid vegetations
315	P	Dec. 28, 1938	Jan. 16, 1939	Mitral vegetation
303	P	Dec. 28, 1938	Jan. 25, 1939	Mitral vegetation
317	P	Dec. 28, 1938	Jan. 28, 1939	Mitral vegetation
322	P	Jan. 17, 1939	Jan. 30, 1939	Negative
338	F	Jan. 27, 1939	Jan. 31, 1939	Negative
313	P	Dec. 28, 1938	Feb. 1, 1939	Tricuspid, aortic, mitral vegetations
341	P	Jan. 27, 1939	Feb. 1, 1939	Negative
328	P	Jan. 17, 1939	Feb. 2, 1939	Negative
336	F	Jan. 27, 1939	Feb. 3, 1939	Negative
307	P	Dec. 28, 1938	Feb. 4, 1939	Tricuspid, mitral vegetations
342	H	Jan. 27, 1939	Feb. 6, 1939	Negative
332	H	Jan. 27, 1939	Feb. 9, 1939	Negative
309	P	Dec. 28, 1938	Feb. 12, 1939	Mitral vegetation, lung
361	F	Feb. 13, 1939	Feb. 20, 1939	Ante mortem thrombus in auricle
331	P	Jan. 17, 1939	Feb. 20, 1939	Mitral and tricuspid vegetations
320	P	Jan. 17, 1939	Feb. 21, 1939	Ante mortem thrombi in auricles
362	F	Feb. 13, 1939	Feb. 21, 1939	Negative
354	P	Jan. 17, 1939	Feb. 23, 1939	Mitral and tricuspid vegetations
368	P	Feb. 13, 1939	Feb. 24, 1939	Negative
326	P	Jan. 17, 1939	Feb. 24, 1939	Mitral vegetations
318	P	Jan. 17, 1939	Feb. 27, 1939	Mitral vegetations, lungs
325	P	Jan. 17, 1939	Mar. 1, 1939	Mitral vegetations, lungs
380	P	Feb. 13, 1939	Mar. 2, 1939	Negative
378	P	Feb. 13, 1939	Mar. 3, 1939	Negative
329	P	Jan. 17, 1939	Mar. 5, 1939	Aortic and mitral vegetations
372	P	Feb. 13, 1939	Mar. 6, 1939	Negative
343	P	Jan. 27, 1939	Mar. 7, 1939	Mitral vegetations, kidney, lungs
365	F	Feb. 13, 1939	Mar. 7, 1939	Negative
376	F	Feb. 13, 1939	Mar. 15, 1939	Mitral vegetations, lungs, spleen

TABLE I (Continued)

Rabbit No.	Strain	Initial inoculation	Death	Significant lesions
310	P	Dec. 28, 1938	Mar. 15, 1939	Mitral lesion
333	H	Jan. 27, 1939	Mar. 16, 1939	Mitral vegetations, lungs
335	H	Jan. 27, 1939	Mar. 18, 1939	Mitral vegetations, kidney
337	F	Jan. 27, 1939	Apr. 5, 1939	Mitral vegetation
373	P	Dec. 28, 1938	Apr. 10, 1939	Decision reserved
399	P	Jan. 17, 1939	Apr. 10, 1939	Decision reserved
468	P	Dec. 5, 1938	Apr. 10, 1939	Decision reserved
306	P	Dec. 28, 1938	Apr. 10, 1939	Decision reserved
311	P	Dec. 28, 1938	Apr. 10, 1939	Decision reserved
319	P	Jan. 17, 1939	Apr. 11, 1939	Decision reserved
323	P	Jan. 17, 1939	Apr. 11, 1939	Decision reserved
381	F	Jan. 27, 1939	Apr. 19, 1939	Mitral vegetations, lungs, spleen, liver

Microscopic sections revealed numerous colonies of streptococci in the vegetations.

Twelve rabbits of a fourth series were inoculated on January 27. Four of these animals received a culture of strain H derived from the blood of a proved case of human endocarditis lenta. All of these died; Rabbit 332 on February 9 after receiving daily intravenous injections from January 27 to February 9 inclusive. The heart of this animal appeared negative at autopsy. The other 3 animals survived for longer periods and all 3 at autopsy showed large endocardial vegetations. Rabbit 334 was the last to die and its protocol may serve to illustrate the entire group. Rabbit 334, weighing 2150 gm., received intravenous injections of a serum-broth culture of strain H, 2 cc. daily on January 27, 28, 30 and 31, and February 1, 2, 3 and 4; 4 cc. daily from February 7 to 23 inclusive; and 6 cc. daily from February 24 to April 15 inclusive. Blood cultures taken at weekly intervals remained persistently negative until April 10. On this date and again on April 13 a blood culture was taken which gave a positive growth. The animal died during the night of April 15 and at autopsy the following morning the spleen was enlarged and the aortic orifice was occupied by massive vegetations.

Four of the rabbits of this fourth series were inoculated with cultures of strain F, likewise from the blood of a patient with proved endocarditis lenta. All are dead. Rabbit 338 died on January 31 after 4 intravenous injections only of 2 cc. each on January 27, 28, 30 and 31. The heart appeared negative. Rab-

bit 336 died on February 3 after 6 injections only. This heart contained a laminated clot attached to the tricuspid valve but microscopic study failed to reveal bacterial colonies. The other 2 rabbits (337 and 381) survived for longer periods and at autopsy showed gross mitral vegetations. Rabbit 381, weighing 2020 gm., received intravenous injections of serum-broth culture of strain F, 2 cc. daily from January 27 to February 18 inclusive; and 4 cc. daily from February 20 to April 17 inclusive. A blood culture taken on January 31 was positive but subsequent weekly blood cultures remained negative until that of April 3, which gave a positive growth. The blood culture taken on April 10 remained sterile but another taken on April 13 became positive. The animal died at 11:40 A.M. April 19, and large vegetations were found in the mitral orifice.

Four of the rabbits of this fourth series were inoculated with serum-broth cultures of strain P. Rabbit 341 died on February 1 after 4 intravenous injections only of 2 cc. each. There was a firm laminated clot in the right ventricle but microscopic examination failed to reveal bacterial colonies. Rabbit 342 died on February 6 after having received 7 intravenous injections of 2 cc. each. There was a small bit of fibrin attached to the mitral valve but bacterial colonies could not be recognized. Rabbit 343 died on March 7 after 31 intravenous injections amounting to a total of 108 cc. of the culture. Blood cultures taken on February 27 and on March 6 were positive. At autopsy the spleen was much enlarged and large vegetations were present in the mitral orifice (Plate 105). Rabbit 340 behaved in a remarkable way. It received 56 injections of the culture, amounting to a total of 208 cc. from February 27 to April 10. Of the weekly blood cultures, those taken on February 6, 27, March 1, 6 and 13 had given a positive growth, but all the others remained sterile. The animal was sacrificed on April 14 and the heart appeared entirely negative in the gross examination.

### DISCUSSION

The table lists 57 animals, of which 27 showed recognizable inflammatory lesions of the endocardium. Nearly all of these lesions were quite large and unmistakable on gross inspection. In fact, there is only one of these 27 accepted as positive in which it was really necessary to await microscopic examination. The



photographs illustrate the characteristics of these lesions, which closely resemble those of actively progressive, malignant endocarditis of man, as seen postmortem. There are 23 animals listed as negative without any searching microscopic study of the heart. It is not improbable that significant positive lesions might be found by thorough microscopic study but this has seemed relatively unimportant at the moment. In 7 animals decision was reserved. All of these were animals subjected to prolonged observation during life in accordance with special therapeutic programs and we wish to make more complete studies of the specimens before discussing them further.

It is already evident that endocarditis lenta, a specific infectious disease of man, can be transmitted to rabbits in a large percentage of experiments by the repeated intravenous injection of large doses of the bacterial culture and that the disease thus produced in the experimental animal exhibits to a large extent the characteristic features of the human disease. It seems, however, that the rabbit possesses a relatively high natural resistance to the infection and we believe, along with Rosenow and Lloyd-Jones, that there is sometimes a real tendency for the lesions in the rabbit to heal unless their development and extension is favored by continued repeated inoculations of the culture or by other depressing influences such as coccidiosis. This apparent balance between the forces of infection and resistance would seem to suggest the rabbit as a valuable experimental animal in which to study the phases of extension and of healing of the lesions and especially for study of the possible influence of various therapeutic measures upon such processes. We are attempting observations of this sort. Meanwhile it is hoped that fellow students in this field may find something helpful in the present report.

#### SUMMARY AND CONCLUSION

1. By repeated intravenous injection of large amounts of pure cultures in serum-broth it has been possible to transmit endocarditis lenta of man to the rabbit.
2. The endocardial lesions in the rabbits have usually been quite large and easily recognizable by gross inspection.
3. Microscopically these lesions resemble those of the human disease. Large colonies of the streptococci are easily found.

4. In the rabbit there is sometimes an evident tendency for these lesions to heal, which suggests that this animal may be of value in testing therapeutic measures against the disease.

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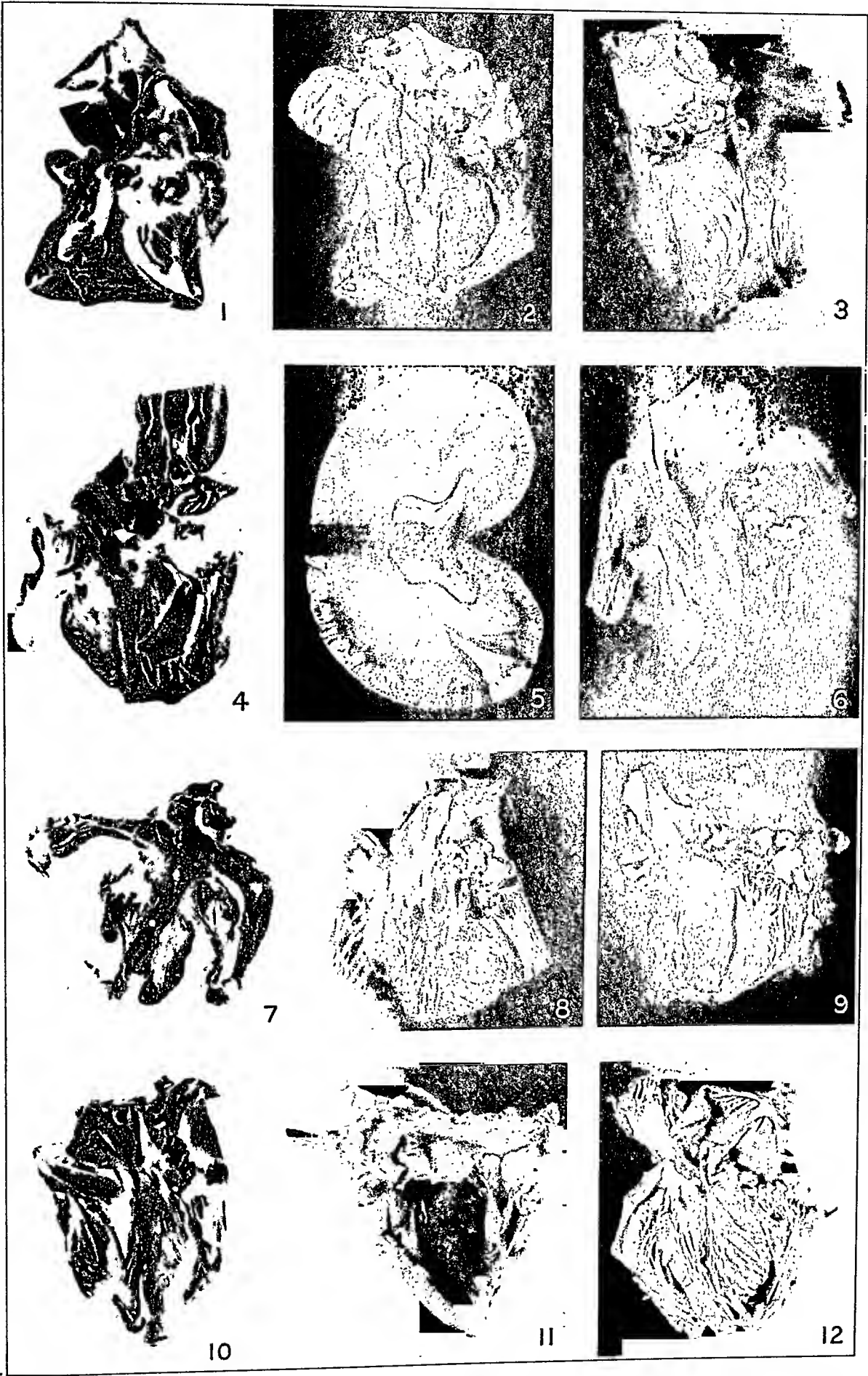
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## DESCRIPTION OF PLATES

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### PLATE 105

- FIG. 1. Heart of Rabbit 303, cut open and showing large mitral vegetations. A small piece removed from this specimen was used to prepare the section for Figs. 15 and 16.
- FIG. 2. Heart of Rabbit 325 with mitral vegetations.
- FIG. 3. Heart of Rabbit 329 with small mitral and extensive aortic vegetations.
- FIG. 4. Heart of Rabbit 313 with tricuspid, mitral and aortic vegetations.
- FIG. 5. Kidney of Rabbit 313 with a recent gross infarct.
- FIG. 6. Heart of Rabbit 381 with large mitral and smaller aortic vegetations. A block was taken through the aortic valve before the photograph was made.
- FIG. 7. Heart of Rabbit 307 with large tricuspid and smaller mitral vegetations.
- FIG. 8. Heart of Rabbit 326 with mitral vegetations.
- FIG. 9. Heart of Rabbit 343 with mitral vegetations.
- FIG. 10. Heart of Rabbit 331 with mitral vegetations and small vegetations near the attachment of the tendinous cord to the tricuspid.
- FIG. 11. Heart of Rabbit 376 with massive mitral vegetations.
- FIG. 12. Heart of Rabbit 318 with mitral vegetations. A bit of black silk, inserted to hold the specimen, is seen near the vegetation at the right border of the picture.



MacNeal, Spence and Wasseen

Experimental Production of Endocarditis Lenta

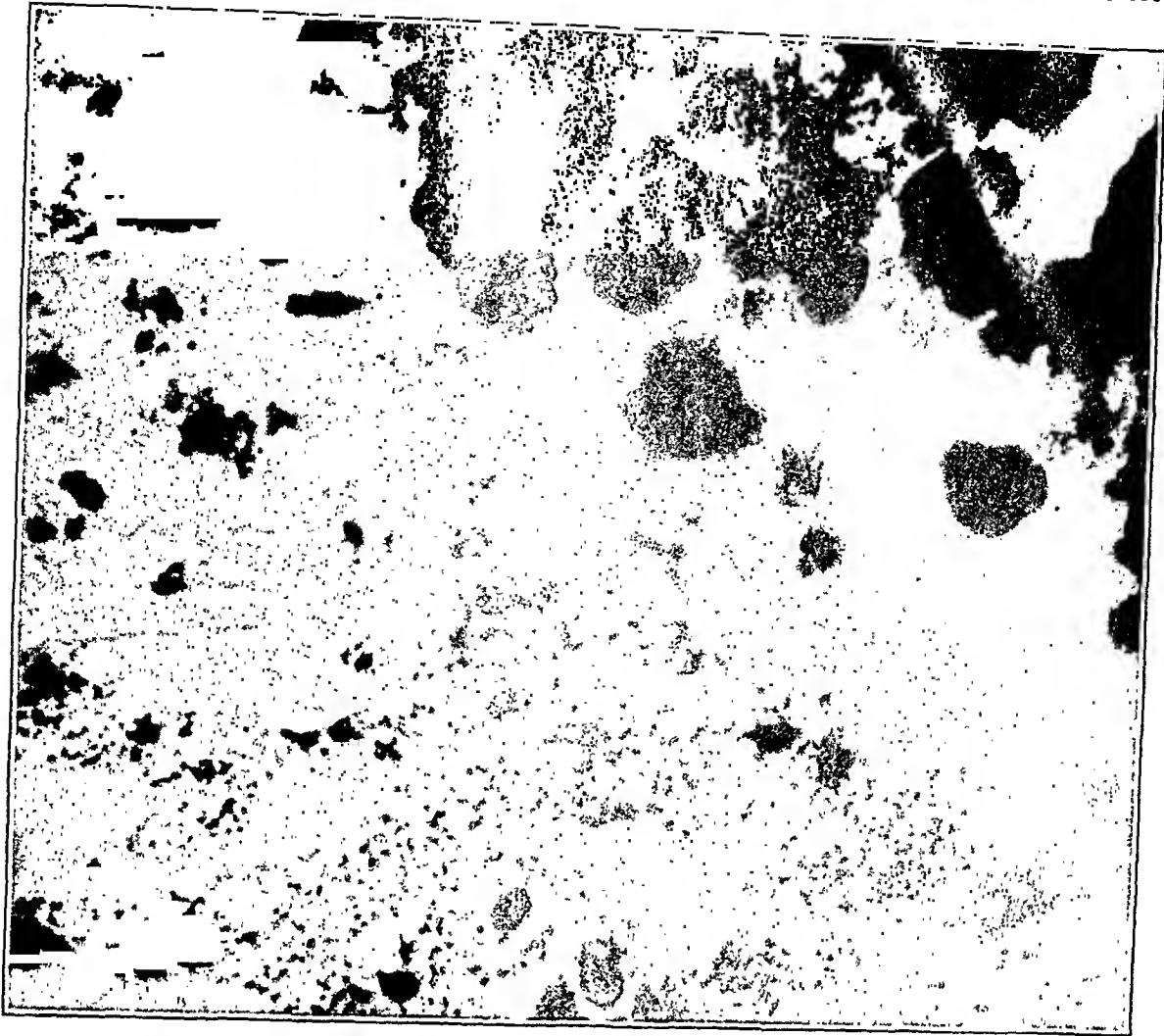
PLATE 106

FIG. 13. Heart of Rabbit 337 with mitral vegetations.

FIG. 14. Heart of Rabbit 354 with mitral and tricuspid vegetations. The latter cannot be clearly seen in the photograph.

FIG. 15. Microphotograph of a section through the mitral vegetation of Rabbit 303 (Fig. 1) stained by the modified Gram method of Brown and Brenn. A portion of the auricular wall is seen above and a portion of the ventricular muscle below. The deformed and thickened mitral flap occupies the center of the figure. It presents a confused intermingling of various elements including large bacterial colonies in necrotic granulation tissue and fibrin, with crumbling at the free margin. Here there are a few erythrocytes included in the fibrin and a red clot clinging to the free surface.

FIG. 16. Microphotograph of a small portion of the same section at high magnification. In this region, near the free margin of the vegetation, is a meshwork of cell-free fibrin containing massive bacterial colonies and, toward the free surface, scattered bacteria and a few well preserved erythrocytes.



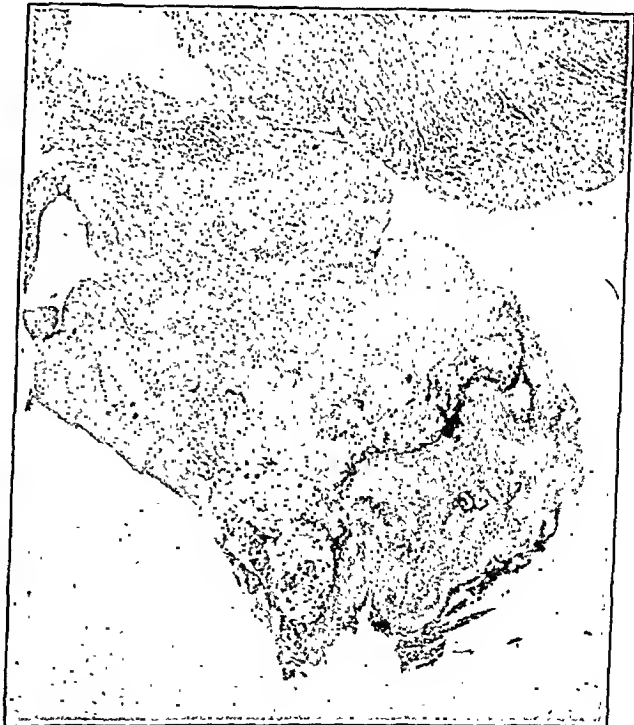
16



14



13



15



STUDY OF THE HISTOLOGICAL CHANGES AND TRANSPLANTATION OF TISSUE SURROUNDING METHYLCHOLANTHRENE PELLETS DURING THE LATENT PERIOD OF TUMOR DEVELOPMENT IN FEMALE C<sub>3</sub>H MICE \*

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When the susceptible tissues of an animal are treated with a carcinogenic hydrocarbon there is a latent period between the first application of the agent and the development of a tumor. The time of tumor induction varies, depending on the agent, the solvent, the tissue treated and the species of test animal employed, and even the genetic constitution of animals of the same species. During this latent period certain of the cells which are exposed to the action of the carcinogenic agent undergo changes antecedent to the development of a frankly malignant process. Since the neoplasms are transplantable in nearly 100 per cent of other mice of the same strain as the animal in which they were originally induced, an experiment was planned by which a comparison could be made between the results of histological examination and transplantation of the tissue around a carcinogenic agent at weekly intervals during the latent period of tumor development when the local cells are assuming the characteristics of autonomous growth.

MATERIALS AND METHODS

The mice employed in this study were of the inbred C<sub>3</sub>H strain obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. They were maintained on a diet of dog chow exclusively, except for the first several days after being received in this laboratory during which time their diet was supplemented with bread and milk. Water was allowed freely at all times. 203 mice were set aside for the experiment. Of these, 81 females aged 5 to 7 months were inoculated subcutaneously into the anterior abdominal wall with methylcholanthrene pellets implanted by means of a trocar and cannula. The pellets contained 5 per

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cent of methylcholanthrene and 95 per cent of cholesterol. The technic followed in making the pellets has been described by Shear.<sup>1</sup> Pellets of cholesterol alone prepared by this method and injected subcutaneously into  $C_3H$  mice do not evoke tumors. In several thousand mice of the  $C_3H$  strain observed under experimental and control conditions in this laboratory during the past several years there has been no instance of the occurrence of a spontaneous sarcoma in the anterior abdominal wall in the experience of the author. The methylcholanthrene-cholesterol pellets were cylindrical in shape and each weighed between 12 mg. and 20 mg. and contained therefore between 0.6 mg. and 1 mg. of methylcholanthrene. At weekly intervals during a period of 98 days 3 pellet animals and in one instance each, 4 and 1 pellet animals a week, 41 in all, were killed and autopsied. The pellet was found in the abdominal wall in 34 of these animals and in the peritoneal cavity in 7. The remaining 40 of the 81 original pellet mice were not used for transplantation purposes because of extensive ulceration of the abdomen, disappearance of the pellet or death. The routine practice was to excise the anterior abdominal wall and spread it out on a cork with the hair side down. In those instances in which the pellet was found in the peritoneal cavity the lesion was widely excised and pinned down on a cork. By means of a pair of iris forceps and fine pointed scissors part of the tissue surrounding the pellet was excised and used for transplantation into other  $C_3H$  mice. The remainder of the tissue with the pellet in place was dropped into fixative and later trimmed in thin pieces which were blocked separately. In obtaining the tissue adjacent to or in contact with the pellet care was exercised to avoid chipping the pellet or including fragments of it in the transplant. However, since the complete absence of methylcholanthrene from the transplant was not proved in any instance in the present experiment, certain of the results are open to the possible interpretation that in their new environment the transplanted cells may have been exposed to the continued effects of concealed hydrocarbon in the transplant. Similarly, certain of the tumors which arose in the recipient mice may possibly have been evoked by particles of the carcinogenic agent accidentally carried over in the graft.

Histologically the character and intensity of the reaction of

the tissues surrounding a methylcholanthrene-cholesterol pellet vary from area to area. Therefore, in order to secure more uniform results it was the practice to select for transplantation samples of any areas of thickening or of otherwise unusual appearance. The excised tissue from each pellet mouse was grafted into the anterior abdominal wall of 3 other strain C<sub>3</sub>H mice by the trocar method. A total of 122 mice, of which 94 were males, was used for transplantation. The mice that received the transplants were examined by palpation each week and any notable changes recorded, including the date on which the successful transplants first showed active growth. The pellet tissue in all cases, and all the transplants which developed into tumors, as well as many specimens of the abdominal wall of mice in which the transplant receded or failed to grow progressively, were examined grossly and microscopically. Most of the tissue preserved for histological study was fixed in Zenker's fluid; occasionally Bouin's or Tellyesniczky's fluid, or dilute formaldehyde solution was used. The tissue was blocked in paraffin, cut at 7  $\mu$ , and stained with hematoxylin and eosin. Other stains also used on occasion were phosphotungstic acid hematoxylin, eosin-methylene blue, Foot's silver stain for reticulum, and Mallory's aniline blue connective tissue stain. Since methylcholanthrene and cholesterol are dissolved in the reagents employed in the paraffin technic, the stained histological sections did not contain the pellet but instead there was a space which corresponded in size and shape to the position of the pellet in the living animal.

Certain terms are used in the following pages which require explanation. The mice into which pellets were injected are referred to as pellet mice or the pellet group; the term positive is applied to those which yielded grafts which grew on transplantation, whereas the opposite is indicated by the designation negative. The mice into which the transplants were inoculated are designated the recipient mice or recipient group, and the terms positive or negative are employed respectively to denote those which did or did not develop a progressively growing tumor at the site of inoculation. In the stained histological sections the space occupied by the pellet in the living animal is referred to as the pellet space.

## CASE HISTORIES AND PROTOCOLS

*At 28 Days:* Transplants of the pellet tissue of Mouse 24 were made into 3 mice (Nos. 70, 74 and 76). Microscopic examination of the pellet tissue revealed that the superficial half of the space was lined by a layer of squamous epithelium which was continuous with the skin surface overlying the pellet. This portion of the skin was thickened and the nearby hair follicles showed atrophy and hyperplasia. A few dilated mammary gland acini near the pellet were lined by flattened squamous epithelium. There were focal and diffuse chronic inflammatory changes with hemorrhage, pigment and numerous basophilic leukocytes. Some small veins were thrombosed. Inside the pellet space were keratin, macrophages and leukocytes in various stages of degeneration and necrosis. Of the 3 mice receiving transplants, 1 (No. 74) was of interest. This animal showed a slight swelling at the site of inoculation a month later which persisted for a few weeks but failed to progress. At autopsy 105 days later there was a small squamous lined cyst on the inner aspect of the abdominal wall at the site of inoculation.

*At 42 Days:* Pieces of the pellet tissue of Mouse 37 were excised and transplanted into 3 mice (Nos. 77, 81 and 92). Histological examination of the abdominal wall containing the pellet revealed an inflammatory process surrounding the pellet space, which occupied a position in the subcutaneous tissue immediately below the panniculus carnosus. Superiorly the space was narrowly separated from the base of the hair bulbs. The deeper portion of the pellet space was in relation to the fat and the abdominal musculature. The pellet space was immediately surrounded by several layers of collagenous fibers containing large vacuolated phagocytic leukocytes and a few elastic fibers. One of the sections included the area immediately adjacent to that which was excised for transplantation. This area was composed of a chronic inflammatory reaction with granulation tissue, polymorphonuclear and mononuclear leukocytes, foreign body giant cells, lymphocytes and fibroblasts, and a few mammary gland acini, some of which showed squamous metaplasia. In this area were small acicular spaces surrounded by degenerated polymorphonuclears. Among the fibroblasts were a few spindle shaped cells which were atypical

in appearance with heavy nuclear walls and large deeply stained aggregations of chromatin material in the nuclei which contained relatively large nucleoli. The thin superficial muscle bundle within the superficial fascia showed atrophy, necrosis and hyperplasia of some of its fibers near the pellet. One small area of the deeper musculature in contact with the pellet was necrotic and showed an inflammatory reaction. There was a decrease in hair follicles and elastic fibers in the skin overlying the pellet. Of the 3 mice inoculated with the pellet tissue 2 (Nos. 77 and 92) lived for 161 days and showed no evidence of growth of the transplant by the end of that time. The remaining mouse (No. 81) showed beginning growth of the transplant at 98 days and progressive enlargement for 49 days, when the animal was killed and showed a spindle cell sarcoma.

*At 49 Days:* Bits of tissue from around the pellet of Mouse 1 were transplanted into 3 mice (Nos. 115, 118 and 119). Microscopically the pellet space, containing masses of necrotic cells and fibrin, lay beneath the panniculus carnosus and above the deep musculature (Fig. 1). It was separated from the latter by fibrofatty tissue containing mammary gland ducts and acini, a few of which showed squamous metaplasia. Individual fibers overlying the pellet space showed necrosis and hyperplasia; occasional muscle fibers contained exceedingly large hyperchromatic nuclei occupying a central position in the cell. Around the pellet space was a collagenous membrane lined internally partly by vacuolated phagocytes and partly by a layer of squamous epithelium. External to this were focal and diffuse chronic inflammatory changes. One area at the lower pole of the pellet space contained numerous atypical hyperchromatic spindle cells. Of the 3 mice that received the transplant, 2 were negative and 1 (No. 119) was positive. One (No. 115) of the negative mice showed beginning enlargement of the transplant at 63 days, this condition being maintained for 2 weeks, after which the lesion regressed. The positive mouse (No. 119) showed beginning growth of the transplant at 70 days. Growth continued steadily for 42 days when the animal was killed and showed a spindle cell sarcoma (Fig. 2).

*At 77 Days:* Bits of tissue surrounding the pellet of Mouse 133 were excised and transplanted into 3 mice (Nos. 179, 184 and

185). Microscopic examination of the lesion showed the pellet space completely surrounded by a connective tissue membrane lined by a thin layer of squamous epithelium over two-thirds of its inner circumference. Outside the pellet space and extending laterally in the subcutaneous tissue was a chronic inflammatory reaction with abundant granulation tissue, hemorrhage, fibrin and necrosis. This area contained isolated hyperchromatic spindle shaped cells with thick nuclear walls within which there was an abundance of coarse chromatin granules. These cells resembled closely those of the tumors which developed from the transplants. Atypical spindle cells showing pronounced mitotic activity were also observed developing in relation to the layer of deep fascia adjacent to the musculature of the body wall separated by some little distance from the capsule of the pellet (Fig. 3). Several nerve fibers were thickened and hyperplastic. A few fibers in the deep musculature and several in the panniculus carnosus showed degenerative and hyperplastic changes. The hair follicles near the pellet space showed diminution and atrophy and a few were enlarged and filled with keratin. The overlying skin showed hyperkeratosis adjacent to the ulcer but no marked thickening. Of the 3 mice which received the transplant 1 (No. 184) died 91 days later without showing any signs of growth at the site of inoculation, while the other 2 developed tumors. One of these mice (No. 185) showed growth of the transplant on the 56th day, with progressive enlargement for 3 weeks when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 179) was found dead 91 days after inoculation and showed a spindle cell sarcoma.

*At 82 Days:* Bits of tissue from a nodule adjacent to the pellet of Mouse 148 were transplanted into 3 male C<sub>3</sub>H mice (Nos. 189, 190 and 192). Microscopic examination of skin in the region of the pellet revealed two nodular lesions near each other in the subcutaneous tissue. One of these was an adenosquamous cell carcinoma and the other a spindle cell sarcoma. Of the 3 mice which received transplants 2 were positive and 1 was negative. Of the 2 positive mice, 1 (No. 190) showed evidence of beginning growth of the transplant at 1 week and continued growth for 49 days when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 189) showed beginning growth

of the transplant at the 9th week and was killed 2 weeks later and showed a spindle cell sarcoma.

Bits of tissue adjacent to the pellet of Mouse 143 were transplanted into 3 mice (Nos. 194, 195 and 196). Histological examination of the pellet tissue revealed a space surrounded by the fibrofatty tissue of the omentum which showed a chronic inflammatory reaction. Within the space were groups of degenerated and necrotic cells, chiefly polymorphonuclear leukocytes and vacuolated mononuclear cells. The inflammatory reaction was circumscribed and contained an abundance of granulation tissue, leukocytes, a few of which were eosinophilic, and mononuclear, small round and spindle cells which appeared to be fibroblasts. Of the 3 mice receiving transplants 2 were positive and 1 was negative. One positive mouse (No. 195) showed beginning growth of the transplant at 21 days. The lesion grew progressively for 84 days when the mouse was killed and showed a spindle cell sarcoma (Fig. 4). The remaining mouse (No. 196) also showed beginning growth of the transplant at 21 days. The lesion enlarged for 126 days when the animal was killed and showed an atypical giant cell sarcoma.

*At 91 Days:* Bits of this tissue from about the pellet of Mouse 130 were implanted into 3 mice (Nos. 198, 201 and 204). Microscopic examination of the pellet lesion showed most of the section to be composed of areolar tissue and a few small fragments of inflammatory tissue. The invading cells were of various types, chiefly mononuclears and small round cells. There were many spindle cells with large, deeply stained, thick walled nuclei containing numerous large chromatin particles. There was considerable collagen in the lesion. A few focal areas of inflammatory reaction occurred about acicular spaces. Of the 3 mice which received transplants, 2 were positive and 1 was negative. One of the positive mice (No. 198) showed beginning growth of the transplant 2 weeks following injection and progressive enlargement for 49 days when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 201) showed beginning growth of the transplant at 42 days, with rapid enlargement of the lesion for 21 days when the animal was killed and showed a spindle cell sarcoma.

*At 98 Days:* Bits of tissue from about a small nodule adjacent

to the pellet of Mouse 134 were implanted into 3 mice (Nos. 206, 207 and 208). Microscopically the small peritoneal nodule was composed of atypical spindle cells, chiefly small round cells and polymorphonuclear leukocytes diffusely distributed throughout the tissue. The nuclei of the spindle shaped cells were oval to rod shaped, a few were round and others elongated with pointed ends. Mitotic figures were extremely numerous, averaging over three per high power field. The nuclei had thickened walls and generally contained a large nucleolus and coarse chromatin particles. The cytoplasm was fibrillar and not very abundant. The cells were usually arranged in more or less distinct parallel bundles and sheets running in various directions, in a few places forming distinct interlacing bundles. The blood vessels, which were not numerous, resembled capillaries or were slit-like and thin walled. There were a few areas of necrosis. Several voluntary muscle fibers with a chain of multiple nuclei in the middle and striations in the fibers were observed in the cellular portion of the nodule. At the periphery the nodule was not well demarcated but instead the peripheral cells infiltrated the surrounding fat. The lesion was suggestive of an early malignant process but a positive diagnosis was not justified on histological grounds. Of the 3 mice which received transplants of the pellet tissue 2 were positive and 1 (No. 208) was negative. Of the 2 positive mice 1 (No. 206) showed beginning growth of the transplant at 14 days and rapid progressive enlargement for 14 days when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 207) likewise developed a spindle cell sarcoma at the site of the tissue inoculation at the 14th day which grew rapidly for 2 weeks when the mouse was killed.

## RESULTS

The results of the experiment are presented in Table I and Table II. All the positive transplants were sarcomas. The case histories and protocols of the mice of the pellet and recipient groups which gave positive results are presented under a separate heading.

A 42 day pellet mouse yielded the earliest fragment of tissue which grew progressively on transplantation and developed into a tumor. The next positive transplant was obtained at 49 days.

From 49 to 77 days there was a lapse of 4 weeks during which none of the transplants gave positive results. Of the 3 pellet mice sacrificed each week from 77 to 91 days, 1 in two instances and 2 in one instance yielded tumor transplants in 2 of the 3 recipient mice used in the respective experiments. Tumors grew in 2 of the 3 recipient mice transplanted with tissue from a pellet mouse at the 98th day. In no instance did all 3 mice receiving transplants excised from around a single pellet develop tumors at the point of

TABLE I

*Results of Transplantation of Tissue Surrounding 5 Per Cent Methylcholanthrene-Cholesterol Pellets in Strain C<sub>3</sub>H Mice*

Number of mice implanted with pellets	Period in days between implantation of pellet and transplantation of tissue	Number of recipient mice	Maximum number of days mice with transplants survived	Number of mice developing tumor from transplant
3	7	9	196	0
3	14	9	189	0
3	21	9	182	0
3	28	9	175	0
3	35	9	168	0
3	42	9	161	1
4	49	12	154	1
3	56	9	105	0
3	63	9	224	0
3	70	9	154	0
3	77	9	112	2
3	82	9	196	4
3	91	8	133	2
1	98	3	126	2

inoculation. Positive results were obtained in 3 of 7 mice in which the pellet was found in the peritoneal cavity at autopsy.

Seventeen mice which received transplants but which were regarded as negative since they failed to develop a tumor, subsequently showed a variable degree of enlargement at the site of inoculation. These cases were distributed as follows: 3 during the first half of the experiment and 14 in the latter half. There was a latent period of from 2 to 11 weeks, averaging 4 weeks, in 8 of these cases during which the transplant remained stationary before enlarging. The duration of enlargement was difficult to estimate in 6 cases, and in the remainder (11 cases) growth continued from 2 to 7 weeks, averaging 4 weeks. In some instances regression was practically complete by the end of the experiment,



the transplant being completely absorbed so that no visible change was found grossly or microscopically at the site of inoculation. In 4 cases a small nodule persisted to the end of the experiment. One of these showed enlargement during the first 3 weeks only and at postmortem examination a nodule 5 by 8 mm. was present in the abdominal wall. On microscopic examination the skin and peritoneum were uninvolved and the lesion consisted of a cyst-like inflammatory area in the subcutaneous tissue, without any evidence of malignancy. Another case showed growth for 1

TABLE II  
*Data on Pellet Tissue which Yielded Successful Transplants*

Number of pellet mouse	Period in days between implantation of pellet and transplantation of tissue	Number of positive recipient mouse	Days after tissue transplantation that progressive growth was first noted	Duration of progressive growth of transplant	Average diameter in mm. of transplant at autopsy
37	42	81	98	49	20
I	49	119	70	42	22
133	77	185	56	21	16
		179	91	?	5
148	82	190	7	49	16
		189	63	14	10
143	82	195	21	84	12
		196	21	126	10
130	91	198	14	49	8
		201	42	21	10
134	98	206	14	14	9
		207	14	14	10

week. At postmortem examination 21 weeks later there was a small yellow elevated nodule 3 by 4 mm. in the subcutaneous tissue. Microscopic examination revealed a small area of fibrosis with no suggestion of malignancy. In the 3rd case the transplant became palpable at 9 weeks, showed enlargement for 2 weeks and then decreased in size but did not completely disappear. At postmortem examination there was a small gray nodule 2 mm. in diameter in the subcutaneous tissue of the abdominal wall. Microscopically this consisted of an encapsulated area of fibrofatty tissue infiltrated with mononuclear leukocytes and lymphocytes but with no evidence of malignancy. In the remaining case a small squamous cell lined cyst was found at the site of inoculation (see Mouse 24 at 28 days).

Six female mice in this experiment developed adenocarcinoma

of the mammary gland. These neoplasms corresponded grossly and microscopically to the mammary carcinomas which develop spontaneously in a high percentage of the females of this strain. Other spontaneous lesions which occurred in some of the mice during the experiment were pneumonia, chronic nephritis, *Klosiella muris* infection of the kidney and prostatic abscess.

### DISCUSSION

This experiment was designed to determine at what point, during the malignant transformation of tissue in contact with a 5 per cent methylcholanthrene-cholesterol pellet implanted subcutaneously into strain C<sub>3</sub>H mice, the cells in the pellet tissue would produce a progressively growing malignant tumor when transplanted subcutaneously into other mice of the same genetic constitution. These results were correlated with the histological changes occurring in the pellet tissue. Employing the same technique, Andervont<sup>2</sup> obtained tumors between 8 and 22 weeks (average 14.5 weeks) in 22 of 25 female strain C<sub>3</sub>H mice injected with 5 per cent methylcholanthrene-cholesterol pellets in the subcutaneous tissue of the right axillary region. In the present study the pellets were allowed to remain in the mice from 1 to 98 days which is approximately the average latent period for tumor development under the conditions of the experiment.

Histological and cytological studies of the premalignant changes in the subcutaneous tissue around a carcinogenic hydrocarbon have shown that atypical cells arise in different locations in relation to the active agent and that these resemble the cells of the fully developed neoplasm (Wolbach,<sup>3</sup> Hval,<sup>4</sup> Rondoni,<sup>5</sup> Lewis,<sup>6,7</sup> and Shear<sup>8</sup>). Shear<sup>1</sup> obtained tumors in recipient mice inoculated with implants excised from the reactive tissue on the 47th day after injection of carcinogenic hydrocarbons and thereafter. In the present experiment little or no correlation could be established between the histological characteristics of the pellet tissue and its successful transplantability in some instances, whereas in others the incidence of atypical cells about the pellet was so high that it was not surprising that transplantation was successful. In control studies in which plain cholesterol pellets were injected into the subcutaneous tissue the lesions showed only a chronic foreign body reaction. In the histological sections of the pellet tissue from

the positive pellet mice in the present experiment atypical cells with characteristics resembling malignant cells were absent in 1 case (Mouse 143 at 82 days), occasionally found in 1 case (Mouse 37 at 42 days) and numerous in 3 cases (Mice Nos. 1, 133 and 130 at 49, 77 and 91 days respectively). In 1 case (Mouse 134 at 98 days) the numerical increase in these cells was so great and their structural arrangement so significant that the lesion in the pellet tissue was suggestive of malignancy. The remaining animal (Mouse 148 at 82 days) showed two small neoplasms near the pellet, one an adenosquamous cell carcinoma, the other a spindle cell sarcoma. Both transplants which developed from grafts obtained from this last mouse were sarcomas. The fact that atypical cells in pellet tissue were observed in profusion in the histological sections from many mice sacrificed before the 42nd day and from several others from 42 to 98 days whose transplants failed to yield tumors in the recipient mice, suggests that other factors in addition to malignant appearing cells about the hydrocarbon are needed for the development of a tumor upon their transplantation into a new host. However, the results of the transplantation studies indicate that malignant changes may be induced in cells exposed to the carcinogenic action of methylcholanthrene before the necessary criteria for the histological recognition of malignancy become fully established.

Positive transplants of pellet tissue were obtained from pellet mice sacrificed at 42 and 49 days and, following a lapse of 4 weeks, at 77, 82, 91 and 98 days. From a study of tumor development in the positive recipient mice it is evident that the property of autonomous growth which cells assume during the evolution of malignancy may be delayed or inhibited when they are separated too early from the carcinogenic agent which initiates this property and are transferred into the new environment afforded by another host. Thus, in several instances in the positive groups the longer the tissue transplant remained in contact with the carcinogenic agent in the pellet mouse, the more apt it was to be successful on transplantation, the less time was required for it to show the first sign of progressive growth, and the more rapid its growth in the host animal. This is illustrated by the pellet mice sacrificed at 42 and 49 days which yielded transplants requiring 98 and 70 days respectively to show beginning growth in the host; whereas in the

case of pellet mice sacrificed at 91 and 98 days the tissue transplants came up at 14 and 14 days respectively. Secondly, the respective transplants from each of the 2 positive pellet mice at 42 and 49 days developed into tumors in only 1 of 3 recipient mice, as compared with 2 of 3 recipient mice receiving tissue inoculations from each of the 5 positive pellet mice from 77 to 98 days. Finally certain individual transplants in the later group (77 to 98 days) grew relatively much more rapidly after their inception than did those in the earlier group (42 to 49 days, Table II). These findings therefore substantiate the view that the adaptation of the transplant to the new host, its growth rate, and its latent period decrease inversely with the length of time the pellet tissue is allowed to remain in contact with the carcinogenic agent.

In most instances when two transplants grew from the same pellet tissue the histological appearance of the two tumors was similar. An exception to this, however, occurred at 82 days. In this experiment Mouse 143 was found to have the pellet in the peritoneal cavity and microscopic examination of the pellet tissue revealed a chronic inflammatory process with relatively few malignant cells. Two of the 3 mice receiving transplants of this pellet tissue developed tumors at the point of implantation. Histological examination of the tumors revealed that 1 mouse had a spindle cell sarcoma composed of relatively uniform spindle shaped cells, while the growth in the other was composed of atypical spindle cells together with numerous tumor giant cells. Since the methylcholanthrene pellet is of sufficient size so that it invariably comes in contact with different tissues along its course, such as blood vessels, nerves, skeletal muscle, connective tissue, skin, peritoneum, and so on, hyperplastic and neoplastic changes may therefore occur in the cells composing various structures.

It is believed that the tumors arising following inoculation of tissue surrounding the pellet were the result of inoculation of malignant cells and not the result of inoculation of small amounts of the carcinogenic agent. In Table II it is seen that in one instance a period of 98 days elapsed between the inoculation of the test material and a palpable growth. Inasmuch as the concentration of methylcholanthrene contained in the inoculated material must have been very minute, if present at all, a period of 98 days is an extremely short latent period for the induction of tumors by

minute quantities of methylcholanthrene. Of 122 animals of the recipient group 110 failed to develop a progressively growing tumor at the site of inoculation of the transplant. If sufficient methylcholanthrene was diffused throughout the tissues from the pellet of Mouse 37 to produce a tumor one would expect to obtain more than 12 tumors when tissues from the area surrounding other pellets were placed in the subcutaneous tissues of 122 other animals.

Seventeen mice in the negative recipient groups exhibited transitory enlargement of the graft at some time or other during the period of observation following transplantation. The causes for this cannot be assigned in each individual case but there would seem to be several factors which might have a bearing on it. The results might have been more homogeneous had the separate transplantation fragments surrounding each pellet been finely ground and pooled before injection. Moreover, in certain cases the inflammatory reaction invariably present in pellet tissue might account for the failure of its cells to become established in the new host. Considering these and perhaps other factors to play a part in this phenomenon it is conceivable that as many as 17 out of 110 mice in the negative recipient group might exhibit temporary local enlargement at the site of inoculation due to inflammatory changes which might also inhibit the establishment of the transplant in the new host. However, when the distribution of these cases over the range of the experiment is considered it is seen that 14 of them occurred among 46 negative animals in the latter half of the experiment from 56 to 98 days inclusive, as against only 3 out of 64 negative animals in the first 49 days. This fact arouses speculation as to whether this transitory enlargement of the transplants may not have been due in part to the temporary growth of cells on the border line of malignancy in an abortive attempt to form a tumor. This hypothesis is supported by the fact that the 14 cases of transitory enlargement occurred at a time when 10 transplants developed into tumors in recipient mice in contrast to the 3 transitory cases of enlargement among a group of only 2 positive transplant mice in the first half of the experiment. Furthermore, if enlargement in this group were due entirely to inflammatory change, it would be expected that this reaction would occur within a relatively short time after transplantation.

This was true in 8 cases in which enlargement followed immediately on transplantation and in 1 case the record is incomplete. However, in the others there was a latent period of 2 weeks in 3 cases, of 3 weeks in 2 cases and of 6, 9 and 11 weeks respectively in 3 cases each. Therefore, in at least 8 of these cases consideration has been given the possibility that the graft contained cells which exhibited temporary signs of proliferation following a definite latent period but failed for one reason or another to develop into fully formed tumors.

### SUMMARY AND CONCLUSIONS

1. Five per cent methylcholanthrene-cholesterol pellets were placed subcutaneously in  $C_3H$  mice. The purpose of the experiment was to compare the results of histological examination and of transplantation of the tissue around a carcinogenic agent at weekly intervals during the latent period of tumor development.

2. Tumor-yielding transplants were obtained from pellet mice sacrificed at 42 and 49 days and following a lapse of 4 weeks at 77, 82, 91 and 98 days.

3. In the histological sections of the pellet tissue which did give rise to progressively growing tumors when transplanted, atypical cells with characteristics resembling malignant cells were present in varying number in the different mice.

4. The results suggest that other factors in addition to the presence of malignant appearing cells about the hydrocarbon are needed for the development of a tumor upon transplantation of pellet tissue into a new host. The results of the transplantation studies indicate that malignant changes may have been induced in cells exposed to the carcinogenic action of methylcholanthrene before the necessary criteria for the histological recognition of malignancy became fully established.

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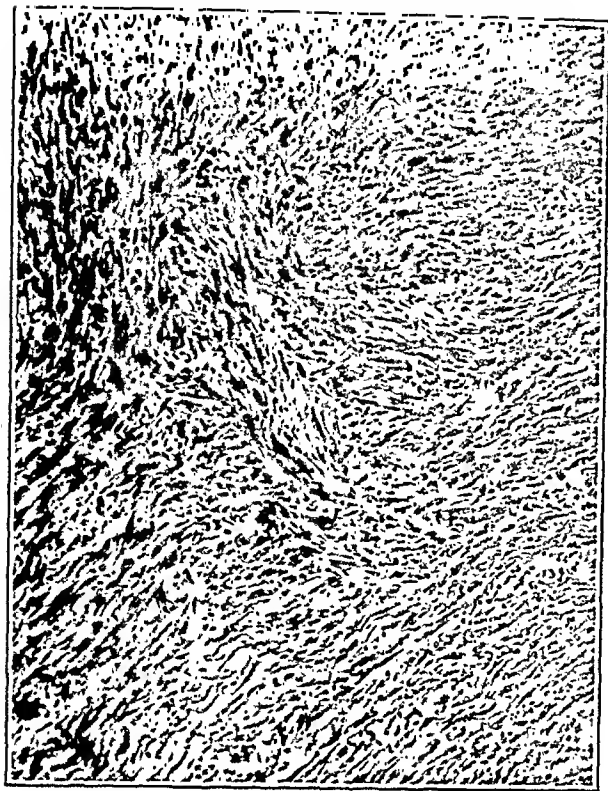
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## DESCRIPTION OF PLATE

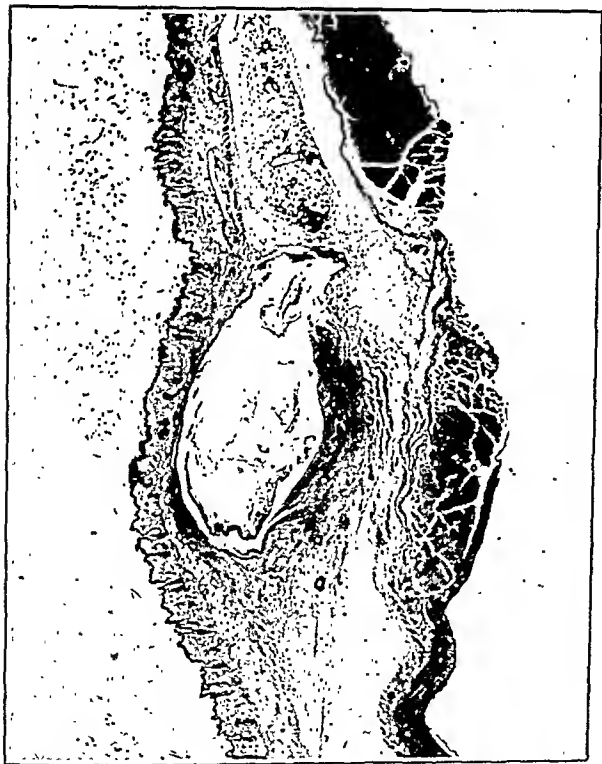
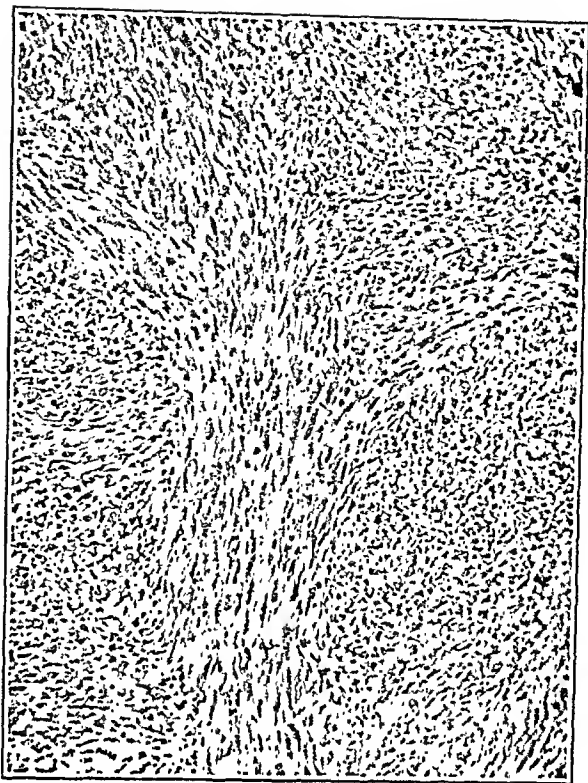
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### PLATE 107

- FIG. 1. Anterior abdominal wall of Mouse 1 inoculated with a methylcholanthrene-cholesterol pellet 49 days previously. The pellet space is in the subcutaneous tissue with the skin above and the deep musculature below. The pellet space is partially surrounded by thickened tissue and on the left border is lined by a thin layer of squamous epithelium which appears partially detached in the illustration. There is a loss of hair follicles over the pellet.  $\times 17$ .
- FIG. 2. Spindle cell sarcoma from Mouse 119. This tumor arose in the abdominal wall at the site of inoculation of a fragment of tissue excised from the pellet capsule of Mouse 1 at 49 days. Histological section of the pellet tissue from Mouse 1 is illustrated in Fig. 1.  $\times 140$ .
- FIG. 3. Mouse 133 injected 77 days previously with a methylcholanthrene-cholesterol pellet. The area is from the deep fascia some little distance removed from the capsule of the pellet. There is an infiltration of a few lymphocytes, mononuclear and polymorphonuclear leukocytes, and proliferation of numerous atypical hyperchromatic spindle cells. Three mitotic figures are present near the center of the illustration.  $\times 350$ .
- FIG. 4. Section of the spindle cell sarcoma of recipient Mouse 195. This tumor grew from implants obtained from the pellet tissue of Mouse 143 which was injected 82 days previously with a methylcholanthrene-cholesterol pellet. The pellet in Mouse 143 was found in the peritoneal cavity rolled up in a mass of omentum.  $\times 140$ .



2



1



3

Stewart

Tissue Surrounding Methylcholanthrene Pellets





## STUDIES ON EXPERIMENTAL RICKETS IN RATS \*

### III. THE BEHAVIOR AND FATE OF THE CARTILAGE REMNANTS IN THE RACHITIC METAPHYSIS

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#### INTRODUCTION

In describing the bones of rachitic infants practically all writers mention a region just below the proliferative zone of the epiphyseal cartilage which is composed of osteoid tissue and marrow interspersed with islands and projecting masses of cartilage. This is the region which is generally known as the rachitic metaphysis (Text-Fig. 1).

There is no general agreement as to the behavior and fate of these cartilage remnants. It is variously stated in textbooks of pathology that they become changed into osteoid, or that they become encased in osteoid, or that their margins change into osteoid. In none of the papers on experimental rickets has this question received much attention. In a paper on rickets in rats Pappenheimer <sup>1</sup> declines to discuss the "difficult question of the direct metaplasia of cartilage into osteoid tissue." Lobeck <sup>2</sup> considers that in experimental rickets in rats these remnants become incorporated into bone trabeculae, the cartilage cells thereby becoming surrounded by bone and subsequently being transformed into bone cells. Park <sup>3</sup> states that these cartilage masses become changed into "pseudo-osteoid, . . . which greatly resembles the osteoid tissue produced by the osteoblasts."

In 1935 <sup>4</sup> we reported briefly on the question but in a later, more extensive paper <sup>5</sup> omitted reference to the matter. We consider the behavior of these masses of considerable interest, not merely as part of the pathological process, but also as having a bearing on the fundamental relations between bone and cartilage.

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This paper presents more mature observations, clearing up some questions and pointing the issue on certain others.

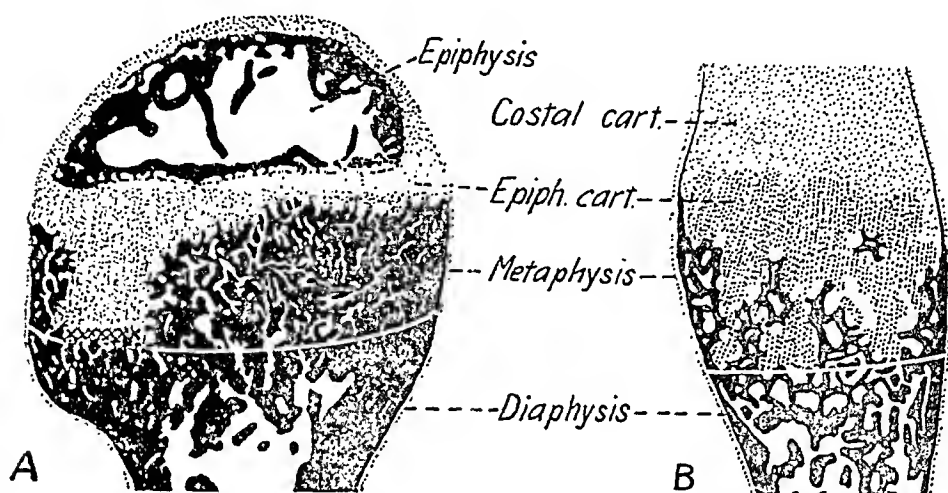
### MATERIAL AND METHODS

This paper is based on continued study of the material used in earlier papers on rickets. Use was made of 125 albino rats; 31 with active rickets, 68 in various healing stages, and 26 healthy animals. Rickets was produced by the Steenbock-Black rachitogenic diet with high calcium, low phosphorus and deficient vitamin D. Healing was induced either by irradiated ergosterol (Viosterol) or cod liver oil. In some of the rats healing occurred spontaneously. The rachitogenic feeding was begun at 4 weeks and the administration of the curative agents at 8 or 9 weeks. Both longitudinal and transverse sections were cut at  $10\mu$  after decalcification in Müller's fluid. Hematoxylin and eosin stains were used. Our main study was on the head of the tibia, with frequent comparisons with other bones. The behavior of the cartilage remnants in the rachitic bones followed essentially the same course whether in active rickets or in early healing stages. The later healing stages furnished important information about the relation between cartilage and osteoid in the metaphysis of rachitic bones.

### THE RACHITIC METAPHYSIS. ITS GENESIS AND NATURE

It is now generally recognized that the rachitic metaphysis is produced by partial removal of the thickened epiphyseal cartilage by the agency of highly vascular marrow from the diaphysis. The process is excellently described by Park.<sup>3</sup> The cartilage cells and the uncalcified cartilage matrix are destroyed with about equal rapidity. The cells degenerate, in the main by pyknosis (Fig. 11), although a few undergo fragmentation and others fading. The invading narrow tongues bore irregular holes in the cartilage, leaving anastomosing cartilage trabeculae of varying thickness, but all large enough to include entire cells as well as matrix. Before long it is seen that there is considerable osteoid associated with the cartilage remnants while at the same time much of the cartilage seems to have disappeared (Fig. 1). The question under consideration is, *does the cartilage change into osteoid?* The location and general extent of the rachitic metaphysis are shown in Text-Fig. 1.

It should be remembered that the cartilage composing these remnants is not of the active growing type, but rather of the kind that makes up the greater part of the rachitic epiphyseal cartilage. The cells are large and are generally considered to be well on the



TEXT-FIG. 1. Drawing showing general structure of bones with well advanced rickets. The open spaces represent marrow. In both bones the greatly thickened epiphyseal cartilage has been partly replaced by the characteristic structures of the rachitic metaphysis. The trabeculae of the metaphysis (shown in black) are composed at the one extreme of persisting portions of the epiphyseal cartilage, and at the other of well developed osteoid, as illustrated in Figs. 1 to 12. Within the irregular trabeculae there runs, with no great distortion, the parallel trabeculae of the epiphyseal cartilage. The region is wholly uncalcified or very nearly so. The trabeculae of the diaphysis and of the epiphysis are composed of persisting prerachitic bone covered with osteoid.

A = Head of the tibia from a white rat, 12 weeks old, with severe rickets. From a tracing over a photograph.  $\times 10$ . XXXX indicates the position of cartilage rejuvenation described on page 731.

B = Costochondral junction in well developed rickets. Adaptation of a figure in a current textbook of pathology, directly comparable to the head of the tibia in Text-Fig. 1 A, except that removal of the cartilage has not been carried so far and the metaphysis is consequently less extensive.

way to disintegration. The cytoplasm is highly vacuolated and faintly basophilic; the nucleus is large, about spherical, and has widely separated chromatin granules. The lacunae have become correspondingly enlarged and the intervening matrix in many places is reduced to thin lamellae (Figs. 1, 2, 3, 9 and 11). In severe rickets this cartilage is without calcification. In both the

main cartilage masses and the smaller remnants the cells have retained their arrangement in longitudinal columns of definitely limited length, except where the weakened cartilage has suffered crushing or distortion by mechanical pressure.

These cartilage trabeculae vary in thickness, the thinner ones including but a single layer of cell columns, while the thicker trabeculae and the intersections have four or five. The following description does not deal with the behavior of the much larger cartilage masses, some of which are always present in the rachitic bones (Text-Fig. 1).

### THE BEHAVIOR AND FATE OF THE CARTILAGE REMNANTS DURING ACTIVE RICKETS

It will be noticed in Figure 1, a typical area from the rachitic metaphysis, that in the upper part of the picture the newer cartilage remnants have the same typically cartilaginous structure as the main cartilage mass from which they spring, whereas those lower down, the progressively modified portions, come to look more and more like osteoid tissue. We will now describe the extent to which cartilage gives place to osteoid and the manner in which this occurs. In this general change we have observed the operation of three general processes — infiltration, envelopment and internal reorganization.

#### *1. The Infiltration of Cartilage with Osteoid*

It is evident that it is only the extreme tips of the marrow masses that have the power of rapid destruction of cartilage. At a short distance below the front the destructive action is limited to the opening of some of the superficial lacunae on the surfaces of the persisting cartilage trabeculae and the destruction of the contained cells, but the formation of osteoid is going on rapidly. These opened lacunae are promptly invaded by osteoblasts, usually one in each lacuna, which have in this region developed in large numbers in the marrow from the cells surrounding the blood vessels. Within each such lacuna there promptly builds in from the periphery toward the center a layer of osteoid matrix, surrounding the enclosed osteoblast (Fig. 3), the deposition continuing until there remains only a small lacuna occupied by the

new osteoid cell. During the filling of the cartilage lacunae with the osteoid matrix the cytoplasmic processes of the osteoblasts, in the usual manner, become surrounded from apex to base by the new matrix, thus forming the canaliculi extending outward from the reduced lacunae (Fig. 10).

The new matrix thus formed within the opened cartilage lacunae usually stains but faintly with eosin, although in some filled lacunae the new matrix shows a deeply staining, granular zone. Thus the superficial portions of the cartilage remnants become infiltrated with osteoid. In the new tissue formed in this manner the pattern of the persisting cartilage matrix shows clearly within the new osteoid formation (Fig. 3).

This tissue might well be called osteoid filled cartilage. It is quite comparable in its mode of formation to the intrachondrial bone described by Bast<sup>6</sup> in the human otic capsule. We are not sure, however, whether any distinction should be made between endochondrial and intrachondrial ossification, inasmuch as both types involve the laying of bone matrix upon a base of cartilage matrix, the only difference being the extent of the cartilage matrix involved. In both types the cartilage matrix persists visibly within the new formation. The concept is nevertheless a useful one and Bast has made a useful contribution in pointing out the exact nature of the process occurring in the otic capsule.

## *2. The Envelopment of Cartilage Remnants by Osteoid*

When the superficial lacunae have been filled by osteoid, as described above, the osteoblasts continue to build more osteoid on that already deposited in the lacunae. In other areas this enveloping layer of osteoid is laid directly on the surface of cartilage trabeculae whose superficial lacunae have not been opened. In either condition the osteoid thus formed may build up to considerable thickness, thus reducing the bore of the marrow canals. This process is most active in a zone of the metaphysis a short distance from the cartilage removal front.

The tissue thus formed is a typical well developed osteoid. It differs from that formed by infiltration of cartilage in that it contains none of the cartilage matrix, that its cells are placed rather at random instead of with a strong tendency to linear arrange-

ment, and that its matrix stains very deeply with eosin, so that it shows as dark areas in photographs (Figs. 1, 2, 4 and 8).

### 3. *The Internal Reorganization of Cartilage*

The foregoing two types of tissue are direct products of the marrow, inasmuch as the cells are derived from the marrow and the matrix is formed under the direct influence of these cells. The type now to be described has a quite different origin, being derived from the cartilage.

We have observed that when the cartilage trabeculae are not more than one or two cell columns in thickness all lacunae are usually promptly opened and filled with osteoid as already described, but when the masses are three or more cells in thickness there are some internal cells that remain unchanged for a longer time (Figs. 1, 2 and 3). These deeper cells are shut off from immediate contact with the marrow by the superficial lacunae which have been filled with osteoid and by the enveloping layer of osteoid. But if the cartilage masses are not too large these internal cells are not wholly beyond the influence of the marrow, as indicated by the behavior now to be described.

After considerable study of many sections we have become convinced that these hypertrophied cartilage cells in unopened lacunae undergo a form of rejuvenation together with a transformation in type. Many of them undergo mitotic division, take on the appearance of osteocytes, and there forms about them within the unopened lacunae a matrix that has the appearance of osteoid matrix such as forms in the superficial opened lacunae. Thus there is produced a tissue which is largely indistinguishable from that formed by infiltration under the influence of osteoblasts from the marrow (Fig. 8). Several lines of evidence support this view:

(a) *Many Lacunae Do Not Become Opened*: After microscopic study of sections, including a series of complete serials through the metaphysis of rachitic bones, it has become definitely apparent that the lacunae under discussion do not become opened. Cross sections of bones have been particularly convincing, inasmuch as such sections enable one to trace the trabeculae of cartilage and the columns of cartilage cells, and thus to determine with certainty which have been opened and which have not (Fig. 2).

(b) *Many of the Cartilage Cells Divide by Mitosis:* Two to four cells are seen in many of the unopened lacunae in these remnants, although only one is present in each lacuna in the general mass of cartilage from which the remnants have come (Figs. 2, 4, 5 and 7). In explanation of this condition mitotic figures of normal appearance are frequently seen in cells which are clearly of the cartilage type. We have observed both first mitoses, making two cells, and second mitoses, making four cells within one lacuna (Fig. 6). The number of dividing cells varies from rat to rat.

(c) *The Cartilage Cells Undergo Transformation into Cells Resembling Osteoid Cells:* This transformation is indicated by the presence of wide gradations of cells in these unopened lacunae, from typical cartilage cells at the one extreme, to cells very much like osteoid cells at the other. These intermediate type cells occur mainly in an intermediate position in the metaphysis. Close to the parent cartilage are found cells only of the cartilage type, while toward the end of the shaft they are mainly of the osteoid type (Fig. 1). This distribution of cell types, when observed repeatedly, leads one to the conclusion that the various intermediate forms are indeed actual intermediate stages leading toward a fairly uniform final type.

The transformation involves change in the staining reaction of the cytoplasm from basophil to acidophil, condensation of the cytoplasm, the development of cytoplasmic processes, and the condensation of the nucleus (Figs. 2, 4, 5 and 7). In general these cells, when fully transformed, are smaller than osteoid cells from the marrow and their cytoplasm is scant and likely to be vacuolated. Their cytoplasmic processes are more slender than those of true osteoid cells. These cytoplasmic processes do not develop by growth but rather are strands of cytoplasm which have retained contact with the capsule of the lacuna while the general mass of cytoplasm underwent condensation. Both kinds of cells are variable and frequently they cannot be distinguished unless it can be seen whether the lacuna has or has not been opened. The transformation begins before the first mitotic division and is continued after the divisions have been completed. Some of the cells, while still of large size, show numerous eosinophilic droplets in the cytoplasm, similar to those described by Pappenheimer<sup>1</sup> in



the ribs of rachitic rats. We do not know their significance but apparently only a small proportion of the cells ever have them (Fig. 5). There are other variations in the details of the transformation but the foregoing account gives a general picture of the process.

(d) *Matrix Forms About These Cells:* While the cartilage cells are undergoing the changes just described the cartilage matrix surrounding the lacunae in which they lie remains undistorted. The lacunae do, however, grow smaller, the change in size being due to the deposition of new matrix within the cartilage lacunae. It appears first as a thin layer in each lacuna, adherent to the capsule, while the cells are yet large and more like cartilage cells than osteocytes in appearance. As the cells grow smaller the matrix increases proportionally (Figs. 4, 5, 6, 7 and 8). The growing matrix progressively surrounds the cytoplasmic processes from apex to base, thus forming canaliculi extending out from the lacunae in which the new osteoid cells lie. When the cartilage cells have divided, producing more than one cell in a cartilage lacuna, some of the new matrix usually forms also between the cells so that each cell thus comes to occupy a separate lacuna. The new matrix in some lacunae stains but faintly with eosin (Fig. 8), while in others it has a zone of granules which stain strongly with eosin (Fig. 7). This deposit is not a thickening of the capsule surrounding the lacuna. The two are of different nature, as indicated by their microscopic appearance, their staining reaction and their behavior during healing of rickets.

The product of these changes is a tissue which is neither cartilage nor osteoid, but with greater resemblance to the latter. Its general staining reaction is pale (Figs. 1 and 8). The cartilage matrix pattern persists. The cells are usually small and look, after their rejuvenation, as if they were once more on the road to degeneration. It must be recognized, although we have not demonstrated it microscopically, that the cytoplasmic processes and the canaliculi in which they lie terminate for each cell at the inner surface of the capsule of the cartilage lacuna, each cell thus failing to communicate by canaliculi with adjacent cells as do the cells of true osteoid. In this respect the tissue resembles cartilage whose cells lack the communicating channels of bone and osteoid.

This tissue resembles the infiltration type of osteoid in that both have the cartilage matrix within the osteoid matrix, and in the usually pale stain of the new matrix. But we are usually not able to distinguish between the two unless the section be cut in a plane to show whether the lacuna has been opened. The two as a group are, however, easily distinguishable from the enveloping osteoid (Fig. 8).

It should be remarked that neither infiltration osteoid nor enveloping osteoid is formed on the large cartilage masses except under special conditions. The surface of such masses usually remains in direct contact with marrow, as if cartilage removal were more or less actively in progress. It was only the smaller masses, not more than about five cells in thickness, which were under the influence of the marrow in such a way as to become covered or filled with osteoid tissue.

In the preceding paragraphs we have described a rejuvenation of cartilage cells, coupled with a transformation into a form and functional activity like that of osteoblasts. Lest the idea of rejuvenation of cartilage cells in rachitic bones may seem unreasonable, or supported by inadequate evidence, we wish again to call attention to a different type of rejuvenation of cartilage cells (Dodds and Cameron<sup>7</sup>) which we have observed frequently and clearly in 43 of the same rats in which we have observed the changes just described. This rejuvenation was recognized also and clearly illustrated by Harris.<sup>8</sup> This process takes place in the main mass of the thickened cartilage close to the end of the diaphysis, the area being marked XXXX in Text-Fig. 1 A. In these areas the hypertrophied cartilage cells become restored to the appearance of cells in healthy growing cartilage: the nucleus becomes smaller and denser, the cytoplasm grows more compact and more sharply contoured, but no cytoplasmic processes develop. The cells undergo mitotic division, giving rise to typical groups of two or four, as in regular cartilage growth, but quite unlike the special flattened cells in the growth zone of the epiphyseal cartilage. The matrix also becomes adjusted to the expanding groups of cells and the characteristic capsules form about the lacunae. The total picture is quite different from the filling process observed in the remnants in the metaphysis. The fact of rejuvenation of hypertrophied cartilage cells is, however, clearly

demonstrated. Comparison of Figures 8 and 9 shows clearly how different are the results of the two kinds of rejuvenation.

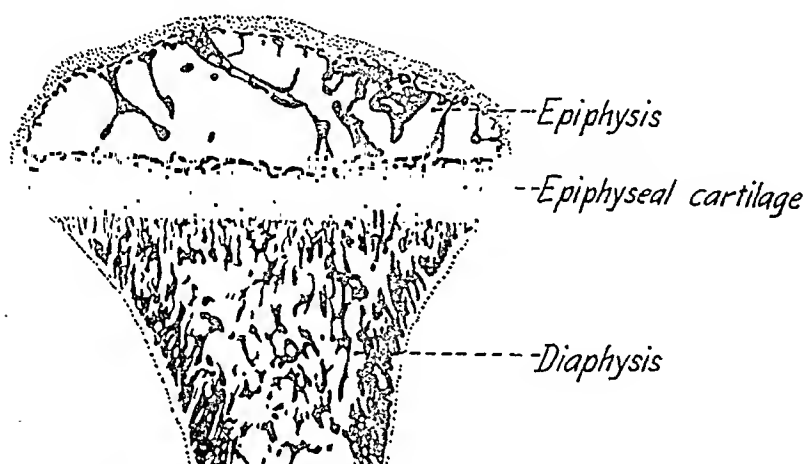
#### CARTILAGE REMNANTS DURING THE HEALING PROCESS

Further light is thrown on the fate of the cartilage remnants by study of healing stages, while calcification is taking place and while the orderly bone trabeculae of the normal spongiosa are taking form in the regions formerly occupied by the chondro-osteoid trabeculae of the rachitic metaphysis.

When calcification is resumed three kinds of calcified matrix are seen in the region of the metaphysis. (1) Calcified cartilage matrix, which stains very deeply and uniformly with hematoxylin and which shows as black in photographs (Fig. 12, C). It follows the pattern of the earlier uncalcified cartilage matrix within the osteoid filler except that the capsules immediately surrounding the lacunae remain uncalcified. The general result is that the longitudinal matrix walls between the columns of cells become calcified, while the transverse partitions between adjacent lacunae within each column remain uncalcified. This is similar to the distribution of calcification in the epiphyseal cartilage of normal bones. (2) Calcified osteoid filler of cartilage lacunae. This portion of the matrix, when calcified, shows clearly about each osteoid cell as a zone of deeply stained granules, separated by a paler zone, the uncalcified capsule, from the dark area of the calcified cartilage matrix (Fig. 12, F). This includes both the infiltration osteoid and that formed by internal reorganization of cartilage tissue. (3) Calcified matrix of enveloping osteoid. This kind of osteoid often occurs in large masses. Calcification appears as clouds of deeply staining granules of varying density (Fig. 12, E).

During the early stages of healing the calcified osteoid filler and the calcified enveloping osteoid are removed, in large part at least, leaving the calcified cartilage matrix as a base for bone deposition. We have made no special study of the fate of the osteoid cells when the matrix is removed. They probably degenerate. Nor have we determined conclusively whether this calcified osteoid is wholly removed or if a small portion persists in the new bone trabeculae. As healing progresses the confused pattern of the chondro-osteoid trabeculae which characterizes the rachitic metaphysis gives place to the rather parallel trabeculae

which characterize the regions in normal bones (Text-Fig. 2). This greater regularity has not been produced by the formation of new trabeculae but rather by the removal of the large amount of osteoid, both enveloping and filling, which during rickets has masked the orientation of the cartilage trabeculae. The cartilage matrix has not been changed into osteoid matrix but much of it has persisted with remarkably little distortion to become the dominant structural element in restoring normal configuration of



TEXT-FIG. 2. Head of the tibia from a white rat 12 weeks old. Complete healing of well developed rickets. From a tracing over a photograph.  $\times 10$ . The somewhat parallel bone trabeculae of the region just below the epiphyseal cartilage have been produced largely from the earlier rachitic metaphysis, the present orientation being determined by the remnants of cartilage matrix which, during rickets, were enclosed in the irregular osteoid masses. This figure serves in most respects also to represent a normal bone which has never shown the rachitic lesion.

trabeculae in the healing bone. After the delay occasioned by the diseased condition the cartilage matrix has performed its normal function in the formation of the early bone trabeculae. This influence of the cartilage matrix is just as effective in the region of the metaphysis, where for a time the cartilage was submerged in osteoid, as it is in regions where the thick cartilage has never taken part in the formation of the metaphysis, except that the distortion due to pressure is somewhat greater in the region of the metaphysis.

We believe that the foregoing paragraphs in large measure answer the question as to the fate of the cartilage remnants in the metaphysis of rachitic rats, although possibly some of the

cells behave in yet other ways than those described. There is every reason to believe that in the main the same conditions will be found to prevail in human rickets when adequate material has been carefully studied.

### DISCUSSION

The foregoing account of the behavior of the cartilage remnants in the rachitic metaphysis portrays, in the main, processes which are quite comparable to those familiar changes in the normal development and growth of bones which result in the deposition of osseous tissue on cartilage remnants. The exceptional feature is the formation of osteoid matrix in unopened lacunae containing rejuvenated cells. We do not know of any description of this process, although several investigators have written rather indefinitely about the metaplasia of cartilage into osteoid tissue.

Our present conclusions are in part different from those expressed in our abstract<sup>4</sup> where it was stated: "We have not observed the deposition of osteoid in unopened cartilage lacunae." At that time we believed that the filling of opened lacunae with osteoid under the influence of osteoblasts from the marrow accounted for the entire mass of cartilage remnants, although soon thereafter our observations brought us to our present conclusions.

Concerning the several conflicting opinions as to the fate of these cartilage masses, it now seems clear that they do become incased in osteoid, that their superficial lacunae become filled with osteoid (not changed into osteoid), and that their internal portions become changed into osteoid only in a very restricted sense. Our observations explain the statement of Lobeck<sup>2</sup> that these remnants become incorporated into bone trabeculae (they become incased in osteoid which later becomes calcified), and that the cartilage cells later are transformed into bone cells (they change into temporary osteoid cells). In all probability these osteoid filled areas are "the transitions which undoubtedly occur between atypical cartilage and osteoid" (Pappenheimer<sup>1</sup>). These rejuvenated and transformed cartilage cells may possibly be the "low-grade bone corpuscles" of Harris<sup>8</sup> in the cartilage islands of rachitic bones, although he gives no adequate description.

Unquestionably this tissue is the "pseudo-osteoid" of Park,<sup>8</sup> although neither he nor any other writer has expressed the same

conception of the genesis and nature of the tissue which we have; *i.e.*, the deposition of new osteoid matrix within the framework of the cartilage matrix. His description leaves no doubt that he has observed the transformation stages of cartilage cells but with different conclusions than ours as to their significance. "We used to think," he writes, "that in rickets actual metaplasia took place, *i.e.*, that cartilage cells, having been reduced to the size and appearance of bone corpuscles, became such. Further studies, however, have convinced us that they actually died." He does not mention mitoses of these cells, nor the other evidences of renewed vitality which we have noted. On the other hand, we concede that their rejuvenation is but transient, inasmuch as they are all seemingly destroyed along with the matrix when healing takes place. We believe that Park's term, "pseudo-osteoid," might very properly be applied to the condition we have described.

In view of the persistent belief that at times cartilage undergoes a direct metaplasia into bone or osteoid, a brief discussion of the points involved in such a change may be in order.

It should be clearly recognized that calcification of the cartilage matrix does not in itself constitute transformation into bone. Such calcification leaves still unchanged the typical structure of the cartilage cells; nor does it cause the matrix to assume the structural nature of bone matrix. Calcification is not the same as ossification, although the two terms are still frequently and wrongly used interchangeably. In normal bone development the matrix of the cartilage becomes calcified in advance of, and as a foundation for, bone deposition, but the cartilage matrix does not become bone although it becomes incased in bone.

When osteoid matrix is formed by osteoblasts in opened lacunae of the cartilage of rachitic bones this cannot be called metaplasia. But it unquestionably is metaplasia when cartilage cells in unopened cartilage lacunae assume the form of osteoid cells, and osteoid matrix is formed about them within the lacunae. The cells have assumed the morphological characteristics of osteocytes, and inasmuch as the matrix formed about them resembles osteoid matrix rather than cartilage, it is clear that they are functioning as osteoblasts. Yet the persistence of the original cartilage matrix within the new osteoid matrix is sufficient to cause the tissue as a whole to fall short of complete metaplasia. So far as we know,

no author has heretofore clearly pointed out the persisting identity of the cartilage matrix within the osteoid of the metaphysis, a point we consider of definite significance. It is thus evident that metaplasia of cartilage involves several elements, each of which must be considered separately.

We may now well consider the four different fates which may befall the cartilage in rachitic bones, with definite relation to regional location and probable external influences: (1) complete and prompt destruction; (2) osteoid infiltration; (3) osteoid rejuvenation; and (4) cartilage rejuvenation. Text-Figure 1 shows the regions now to be considered. In this connection there appears to be justification for the belief of Park <sup>3</sup> that "the cartilage cell cannot be exposed to the influences of blood and remain cartilage."

Cartilage is typically an avascular tissue. Prompt destruction of cartilage cells and matrix takes place at the most advanced portions of the marrow tongues where the raw eroded surface of the cartilage is in immediate contact with the marrow. The marrow at the very front is composed almost wholly of endothelial channels containing blood (Fig. 11). Sometimes, but not often, there are a few primitive connective tissue cells in advance of the vessels. Often there are erythrocytes outside the vessels in the recently opened cartilage lacunae. It would seem indeed that, as assumed by several recent writers, the vascular tissue is the active agent of cartilage destruction. It will be recalled in this connection that in the cartilage removal zone of the normal epiphyseal cartilage the blood channels are in intimate contact with the cartilage, but succeed in destroying only the uncalcified portion, *i.e.*, the cells and the transverse walls within cell columns. The calcified portions apparently are destroyed only by the osteoclasts (Dodds <sup>9, 10</sup>).

Connective tissue cells become abundant between the blood vessels and the cartilage masses at a short distance back from the front of the marrow tongues. Some of these cells soon become osteoblasts on the surface of the cartilage. This is the region of "incomplete exposure to blood" (Park <sup>3</sup>). In such regions cartilage removal ceases except for the opening of the more superficial lacunae and the destruction of their cells. Extensive deposition of osteoid occurs both in the opened lacunae and on the general surface of the cartilage masses. Moreover, it is in such regions that there takes place the transformation of the deeper cartilage

cells, and the deposition of osteoid matrix in unopened lacunae. It appears that the osteoid forming influence of the marrow is not limited to the surfaces in immediate contact with osteoblasts, but also extends to the inner cells of the cartilage remnants, causing them to behave like osteoblasts.

Our observations of the transformation of these cartilage masses exemplify the statement of Park <sup>3</sup> that "the closer to blood vessels, the more the condition resembles osteoid and the further away the more numerous are the cartilage cells which still retain normal features."

After considerable osteoid has been formed on the cartilage remnants and the marrow spaces have become smaller the osteoblasts disappear and osteoid formation ceases. By this time the transformation of the deeper parts of the cartilage masses has also become well advanced.

During active rickets the interior of the large masses of cartilage is clearly beyond the range of influence of the marrow, but it is of interest that in such cartilage, near the cartilage-shaft junction, there often takes place the type of cartilage rejuvenation which produces cartilage. This region is not immediately adjacent to any large masses of marrow but it is not far away from smaller marrow channels in the diaphysis whence there undoubtedly diffuse substances into the adjacent cartilage. One would like to know whether the diffusing substances are different in nature from those reaching the cartilage remnants in the metaphysis, or if they are only less abundant so that cartilage instead of osteoid results from the rejuvenation.

The four types of cartilage reaction just enumerated seem to be determined by the marrow, as if there were a definite gradation or zonation of influence. In part this influence must be through the power of the blood to carry nutrient materials to the tissues and to carry away wastes. But other factors must also be involved such as specific action of various cells in the marrow — the vascular elements, the osteoblasts, the osteoclasts, and probably other unidentified cells. The whole complex of processes is highly involved and embraces the entire scope of the differentiation of the mesenchymal derivatives which participate in bone formation.

In this connection one might ask whether the influence of the marrow is first on the changing cartilage cells and through them



on the matrix, or if both cells and matrix show direct influence from the marrow. Obscure as are these points, they are no more so than are the intimate questions of normal ossification, which in considerable measure still await the attention of the anatomist and the biochemist.

### SUMMARY

The cartilage remnants in the rachitic metaphysis of experimental rats were observed to behave as follows:

1. Considerable osteoid is deposited on the surfaces of the cartilage remnants — osteoid envelopment of cartilage.

2. Osteoid is deposited in opened lacunae near the surface of these cartilage remnants by the action of osteoblasts which enter the opened lacunae from the adjacent marrow — osteoid infiltration of cartilage.

3. Cartilage cells farther below the surface of the remnants undergo rejuvenation; many of them divide mitotically and assume the form of osteocytes. While this change is going on, uncalcified osteoid matrix is deposited about them in the unopened lacunae — internal osteoid reorganization of cartilage.

4. In the foregoing internal reorganization process the cartilage cells undergo a metaplasia into osteocyte-like form, but the matrix of the cartilage is not changed. It persists, to become calcified during the healing of rickets and to serve as a base for the deposition of bone in the formation of the trabeculae of the restored bone.

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### DESCRIPTION OF PLATES

In all photographs the uncalcified cartilage matrix surrounding the cartilage lacunae shows darker than the uncalcified osteoid matrix which has filled the cartilage lacunae. The enveloping eosinophilic osteoid shows as definitely darker than the osteoid which fills the cartilage lacunae. All photographs are from the head of the tibia. All figures except Figure 12 show active rickets.

1 = Cartilage matrix; 2 = osteoid matrix in opened lacuna, infiltration osteoid; 3 = osteoid matrix in unopened lacuna, transformation osteoid; 4 = space between cell and matrix; 5 = osteoblast or osteoid cell in opened lacuna, infiltration osteoid; 6 = enveloping osteoid; 7 = osteoblast; 8 = hypertrophied cartilage cell; 9 = cartilage cell in transformation to osteoid cell form in unopened lacuna; and 10 = cartilage cell which has been rejuvenated as cartilage cell.

p = Cartilage cell undergoing pyknosis (Fig. 11); e = erythrocytes in advance of nucleated marrow cells at invasion front (Fig. 11); C = calcified cartilage matrix (Fig. 12); F = calcified osteoid filler of cartilage lacunae (Fig. 12); E = calcified enveloping osteoid (Fig. 12); and UE = uncalcified enveloping osteoid (Fig. 12).

### PLATE 108

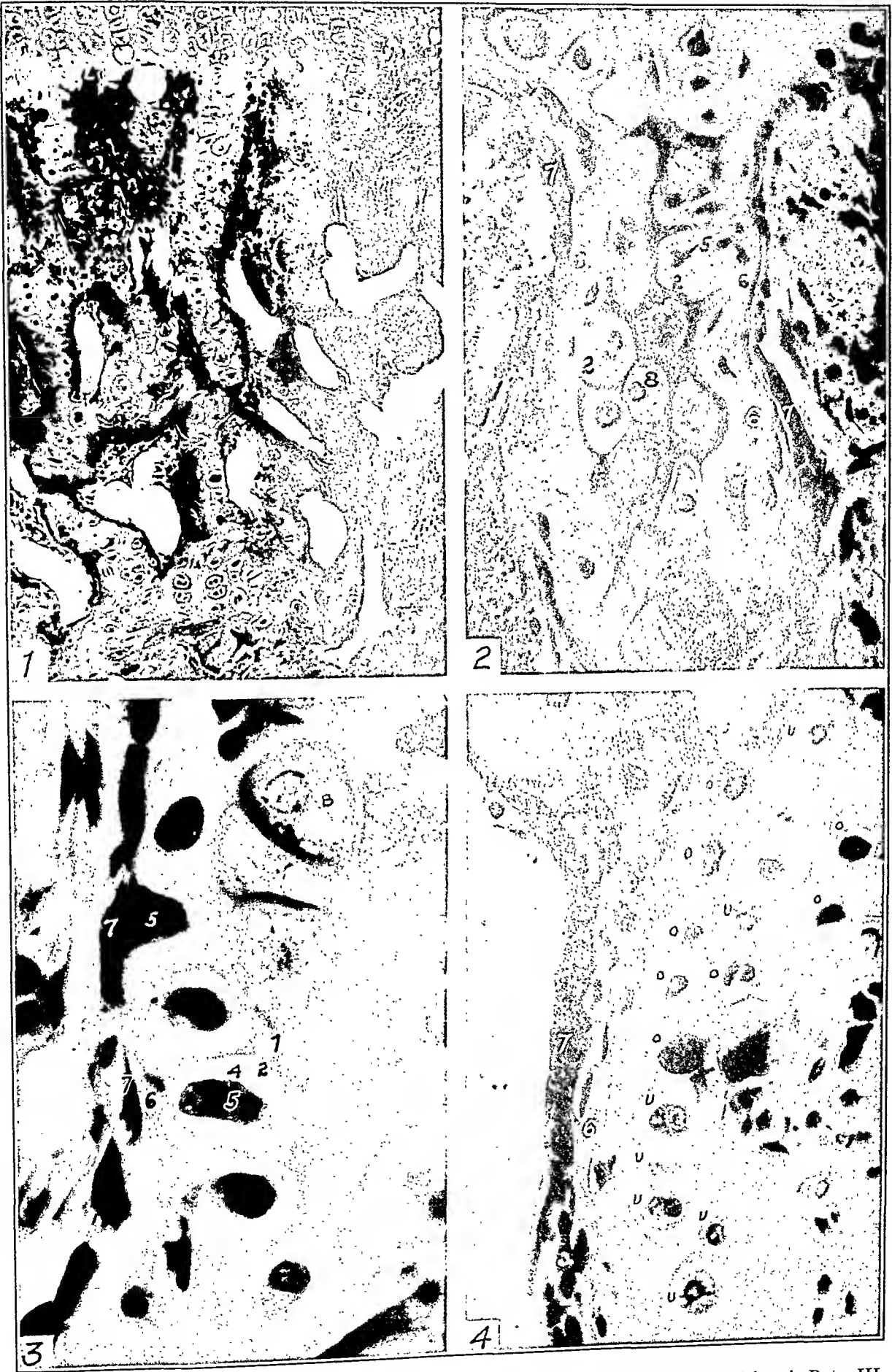
FIG. 1. Edge of cartilage and part of metaphysis of a rachitic rat 8 weeks old. Projecting from the general cartilage mass are the persisting trabeculae of the cartilage, composed of the usual hypertrophied cells occupying lacunae separated usually by only thin walls of cartilage matrix. Farther from the cartilage mass these cartilage trabeculae give place to the different forms of osteoid. The true osteoid (enveloping osteoid) shows darkly in the photograph; osteoid filling the cartilage lacunae is usually lighter

in color. Some marrow spaces are empty because portions of the section have dropped out.  $\times 100$ .

FIG. 2. Portion of a cartilage trabecula from a rachitic rat 8 weeks old showing early phase of transformation. The cartilage matrix is not calcified although it is deeply stained. On the surface is a layer of enveloping osteoid (6) being built up by the action of the osteoblasts (7). A few of the superficial cartilage lacunae have been opened and osteoid deposited (2). Most of the cartilage lacunae have not been opened (8). Some cartilage cells are still unchanged, others are in various early transformation stages, and some have divided. A little osteoid filler has been formed in a few unopened lacunae (not clearly seen in photograph).  $\times 450$ .

FIG. 3. Margin of cartilage trabecula from a rachitic rat 8 weeks old. The superficial lacunae have been opened and are being filled with osteoid (2). Osteoblasts from the marrow occupy the opened lacunae and also lie on the general surface of the new osteoid (5, 7). A few unchanged cartilage cells remain within the mass (8).  $\times 950$ .

FIG. 4. Margin of a cartilage remnant from a rachitic rat 8 weeks old showing osteoblasts on the surface (7) and depositing enveloping osteoid (6). This section shows a series of transformation stages of cartilage cells into osteoid cell form in unopened lacunae (U). Some of the cartilage cells have divided. Osteoid filler is present in varying amounts in these lacunae, even where the cartilage cells have changed but little. Other lacunae have been opened and are now occupied by osteoid filler and by osteoid cells from the marrow (O).  $\times 450$ .



Experimental Rickets in Rats. III

FIG. 5. Transforming cartilage cells in unopened lacuna from a rachitic rat 8 weeks old. The first mitosis has been completed. These cells show the eosinophilic droplets (dark spots) in the cytoplasm. Some osteoid filler has already been formed about the cells in the lacuna (3).  $\times 950$ .

FIG. 6. Second mitosis of a cartilage cell in an unopened lacuna from a rachitic rat 8 weeks old. The other cell has completed the second division. A thin layer of osteoid filler is seen in this lacuna (3).  $\times 950$ .

FIG. 7. Showing transformation of cartilage cells and osteoid filling more advanced than in Figure 4. From a rachitic rat 7 weeks old. Most of the cartilage cells have divided. Considerable osteoid matrix has been formed in the unopened lacunae (3). In some the inner zone of new matrix shows eosinophilic granules (dark color), a condition sometimes seen in the filler of both open and closed cartilage lacunae. One of these lacunae has been opened (2). Enveloping osteoid is also seen in some areas (6). This area is from the lower part of Figure 1.  $\times 450$ .

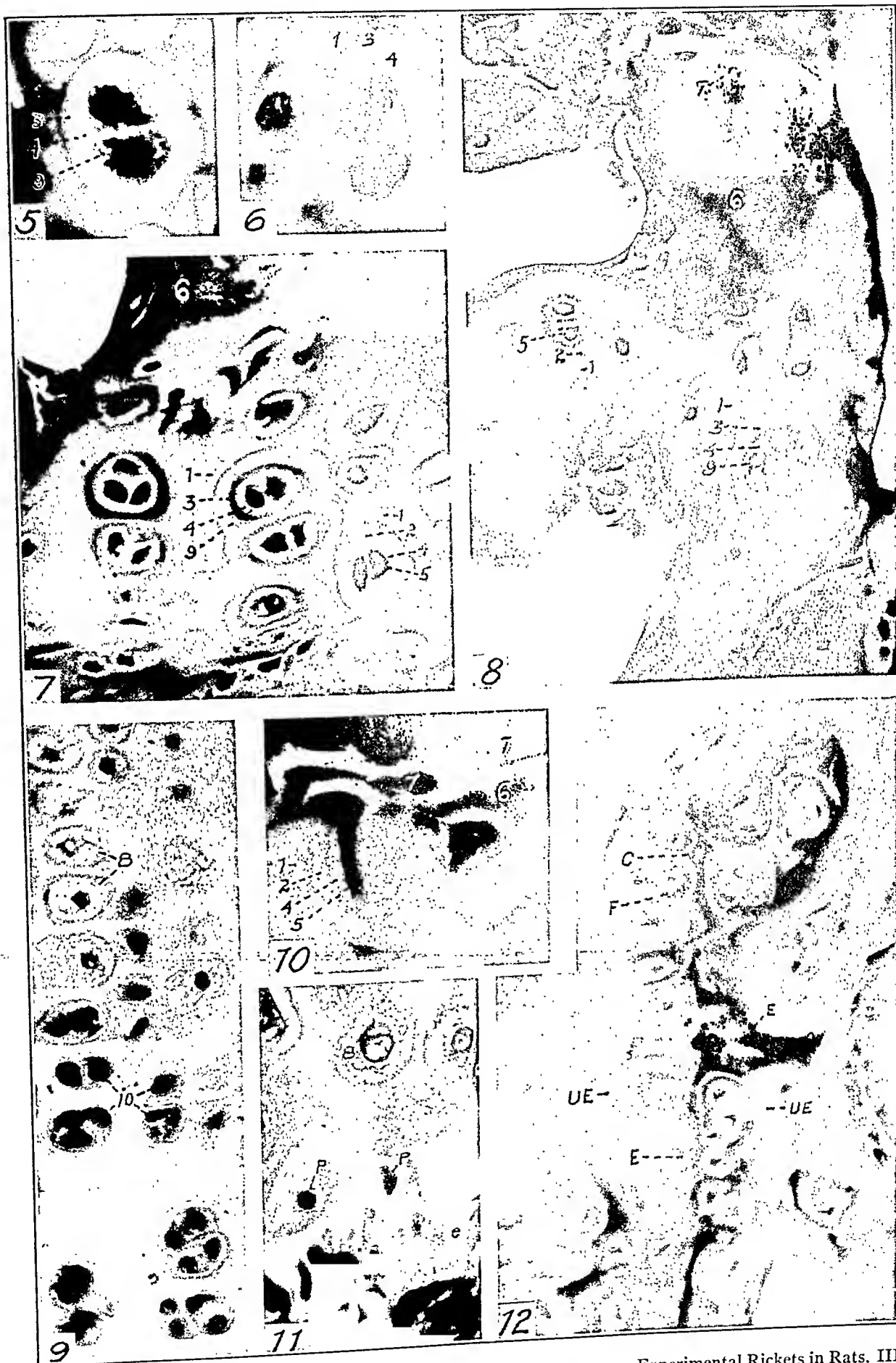
FIG. 8. Portion of a cartilage remnant showing well advanced osteoid filling of an unopened lacunae (light areas) from a rachitic rat 8 weeks old. Shows adjacent enveloping osteoid (6). Osteoblasts (7) are still laying enveloping osteoid in some regions. In the filled area the cartilage matrix pattern (1) shows clearly, as well as the osteoid filler of the lacunae (3). The cells of this area do not appear as vigorous as in the adjacent enveloping osteoid—a common condition. At least one of the lacunae in this field has been filled by infiltration (2).  $\times 450$ .

FIG. 9. Cartilage rejuvenation in the deeper part of the thick cartilage adjacent to the end of the shaft. From a rachitic rat 10 weeks old. Shows ordinary hypertrophied cartilage cells (8) and others in various stages of rejuvenation (10). Some of the cells have divided once, others twice. The rejuvenated cells appear and act like cartilage cells, producing a tissue which looks like cartilage but not like osteoid. It is very different from the light area in Figure 8.  $\times 450$ .

FIG. 10. Margin of cartilage remnant from a rachitic rat 8 weeks old. Shows two osteoblasts (5) about which the osteoid matrix is still being deposited in the lacunae (2). These cells show cytoplasmic processes extending into the canaliculi in the newly formed osteoid matrix. Enveloping osteoid (6) is also being deposited by osteoblasts (7) above these cells on the general surface.  $\times 950$ .

FIG. 11. Edge of cartilage where it is being destroyed by marrow. From a rachitic rat 7 weeks old. Shows the usual hypertrophied cartilage cells (8). Two of these are undergoing pyknosis (p), one in an unopened, the other in an opened lacuna. The marrow shows erythrocytes (e) at the very front followed closely by various nucleated marrow cells.  $\times 450$ .

FIG. 12. Portion of a trabecula in metaphysis when healing is well under way. From a rachitic rat 9 weeks old. The calcified areas may be differentiated as follows: calcified cartilage matrix, deep and uniform black (C); calcified osteoid filler, dark granular areas surrounding cells (F); and calcified enveloping osteoid, dark granules in varying density (E). Portions are still uncalcified and lightly colored in the photograph (UE). This area suggests Figure 2 and others.  $\times 450$ .



### Experimental Rickets in Rats. III



## ESSENTIAL AND PAROXYSMAL HYPERTENSION, CONTRASTED BY CASE REPORTS \*

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Articles dealing with essential hypertension are omnipresent in current medical literature. A number of cases of paroxysmal hypertension have been reported and another isolated example would add nothing to our knowledge of this subject. It is my intention to contrast essential and paroxysmal hypertension, hoping thereby to simplify their differential diagnosis.

Essential hypertension of the so-called benign and malignant phase is by reason of its secondary pathology one of the most frequently encountered lethal factors, accounting for approximately 11.4 per cent of all deaths.† Paroxysmal hypertension was the lethal factor in less than 0.1 per cent of the same series of cases. Paroxysmal hypertension is a clear-cut pathological-physiological entity representing the physiological response to neoplastic hyperplasia of normally functioning cells, the pheochromocytoma cells of the adrenal medullary parenchyma. On the other hand, the causative factor of essential hypertension of the benign type is a matter of speculation, still resting on hypotheses, none of which is entirely tenable. In the second part of this paper (dealing with paroxysmal hypertension) a rather detailed consideration will be accorded the adrenal gland because it constitutes one of the many hypothetical etiological factors of essential hypertension, as well as being the actual site of the known causal pathology of paroxysmal hypertension.

### PART I

#### ESSENTIAL HYPERTENSION OF THE BENIGN TYPE

Medical literature dealing with essential hypertension includes the life work of universally recognized specialists in all

\* Received for publication July 27, 1939.

† A tentative figure based on incompletely classified forms of chronic cardiovascular disease encountered among 1105 autopsies performed by the author during his tours of duty.



branches of our profession. Almost all the valuable work now being accomplished on this subject is along medical, surgical and experimental chemical-physiological lines, and it would appear that too little attention is focussed on the data included in autopsy protocols.

The term essential hypertension has been generally accepted since Allbutt<sup>1</sup> in 1895 recognized the occurrence of isolated hypertension by following patients with increased blood pressure over periods of years. I would define essential hypertension as the manifestation of sustained high blood pressure without recognizable cause, although later secondary cardiac, cerebral and renal vascular changes occur. Naturally all individuals with essential hypertension of the benign type do not succumb to this ailment and the secondary vascular changes may merge into those of the senile form. Logically the term essential hypertension should be limited to the condition usually referred to as essential hypertension of the benign type.

In selecting the cases to be reported, 1 case essential hypertension of the benign type, the 2nd case one of paroxysmal hypertension, I was able to find records of 2 patients who presented much in common. Both were white males of the same age at the time of death (45 years); both were army officers, one a member of the Medical Corps, the other of the Veterinary Corps; and both died as the result of cerebral hemorrhage. I was enabled through the courtesy of the Office of the Surgeon General to secure the necessary data to prepare graphs illustrating the blood pressure readings of both officers from the time of their entry into the Service to death (Graphs 1 and 2).

### *Case 1. Essential Hypertension of the Benign Type*

*Clinical History:* The patient, P. McN., was a white male, aged 45 years at the time of death. He was a moderate user of alcohol and smoked in excess, approximately 50 cigarettes daily. His father died at 60 years of age from cardiovascular disease with diabetes; his maternal grandmother died at 60 years from apoplexy; and a paternal aunt is reported to have had a blood pressure above 200 mm. Hg. for a number of years.

The past illnesses of the patient included mumps at the age of 22 years, complicated by orchitis. He had had mild influenza several times and several attacks of tonsillitis. The Wassermann and Kahn reactions were always negative.

In May, 1931, during the routine annual physical examination of this offi-

cer, a trace of albumin and hyaline casts were found in the urine. During 1932 the patient had repeated hematuria due to associated nephrolithiasis (see below). In December, 1932, he had severe epistaxis which required packing to control. The blood pressure readings are depicted in Graph 1.

The patient was admitted to the Walter Reed General Hospital, Washington, D. C., Nov. 14, 1933. At the time of admission he was ambulatory, afebrile and in fairly good general condition. The blood pressure on admission to the hospital was 220/140, and the pulse pressure 80 mm. Hg. The eyegrounds revealed marked angiosclerosis with scarring and recent small retinal hemorrhages. The blood count showed 3,830,000 erythrocytes; hemoglobin 75 per cent; total leukocyte count 8000; and the polymorphonuclears 69 per cent. The urea nitrogen was 20 mg. per cent, the blood sugar 108 mg. per cent. The basal metabolic rate was plus 2. The electrocardiogram showed left axis deviation and inverted T waves in lead I. Roentgenograms revealed widening of the aortic arch and left ventricular hypertrophy; the lungs were negative and the kidneys showed dense shadows in both renal areas. The specific gravity of the urine ranged from 1.006 to 1.027; all specimens of urine presented 1 to 2 plus albumin, sugar negative, many leukocytes and occasionally many erythrocytes. The urinary findings are in part accounted for by the complicating nephrolithiasis.

On Feb. 8, 1934, the patient complained of severe basal headache. On February 10 he had epistaxis. Muscular twitchings were noted and he became unconscious for one-half an hour. On February 11 the blood pressure was 270/170, and the pulse pressure 100 mm. Hg. A spinal puncture was made and clear fluid obtained. On February 23 at 12 midnight he had a convulsion and became unconscious for a short time. The blood pressure was then 235/143 mm. Hg. and flaccid paralysis of the right lower extremity supervened. A second convulsion accompanied by unconsciousness came on at 1.30 A.M. At 2.00 A.M. there was a third convulsion, and a fourth at 2.45 A.M. Flaccid paralysis of the right upper and lower extremities was noted. The left pupil was dilated and the right pupil was contracted to pin-point size. He remained unconscious until death, which occurred at 2.45 P.M., Feb. 24, 1934.

### *Postmortem Examination\**

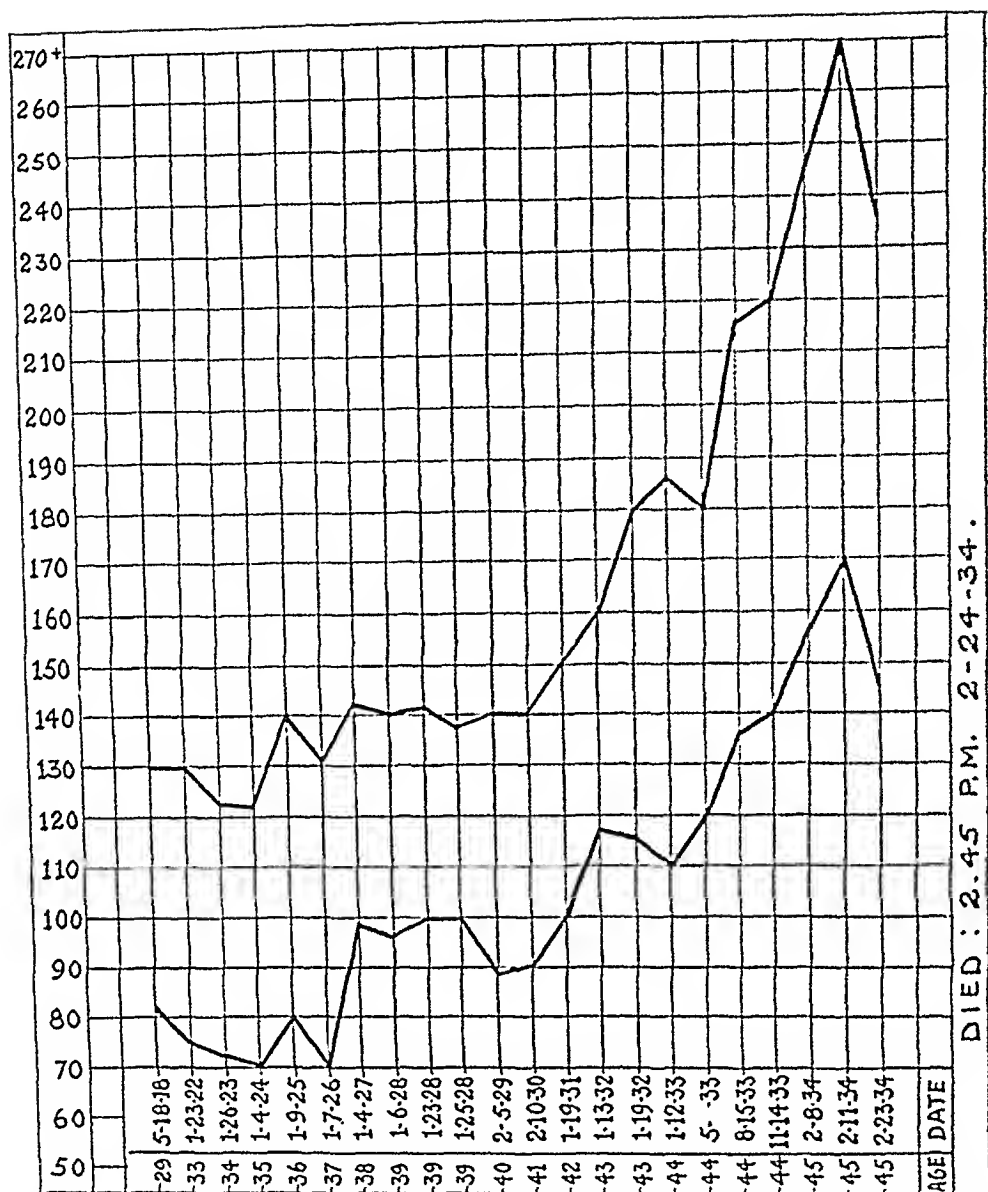
The body length was 175 cm., the weight approximately 61 Kg.

*Heart:* The heart weighed 550 gm. The wall of the right ventricle measured 7 mm. in thickness, the wall of left ventricle 2.8 cm. The aortic ring was 7.7 cm. in circumference. The coronary arteries showed moderate intimal thickening; the lumens were patent and the contents fluid blood.

*Aorta:* The aortic arch measured 6.5 cm. in circumference, the thoracic aorta 5.5 cm., and the abdominal aorta 4 cm. The aortic intima was diffusely thickened and presented plaques of fat imbibition, but no calcification or ulceration was observed.

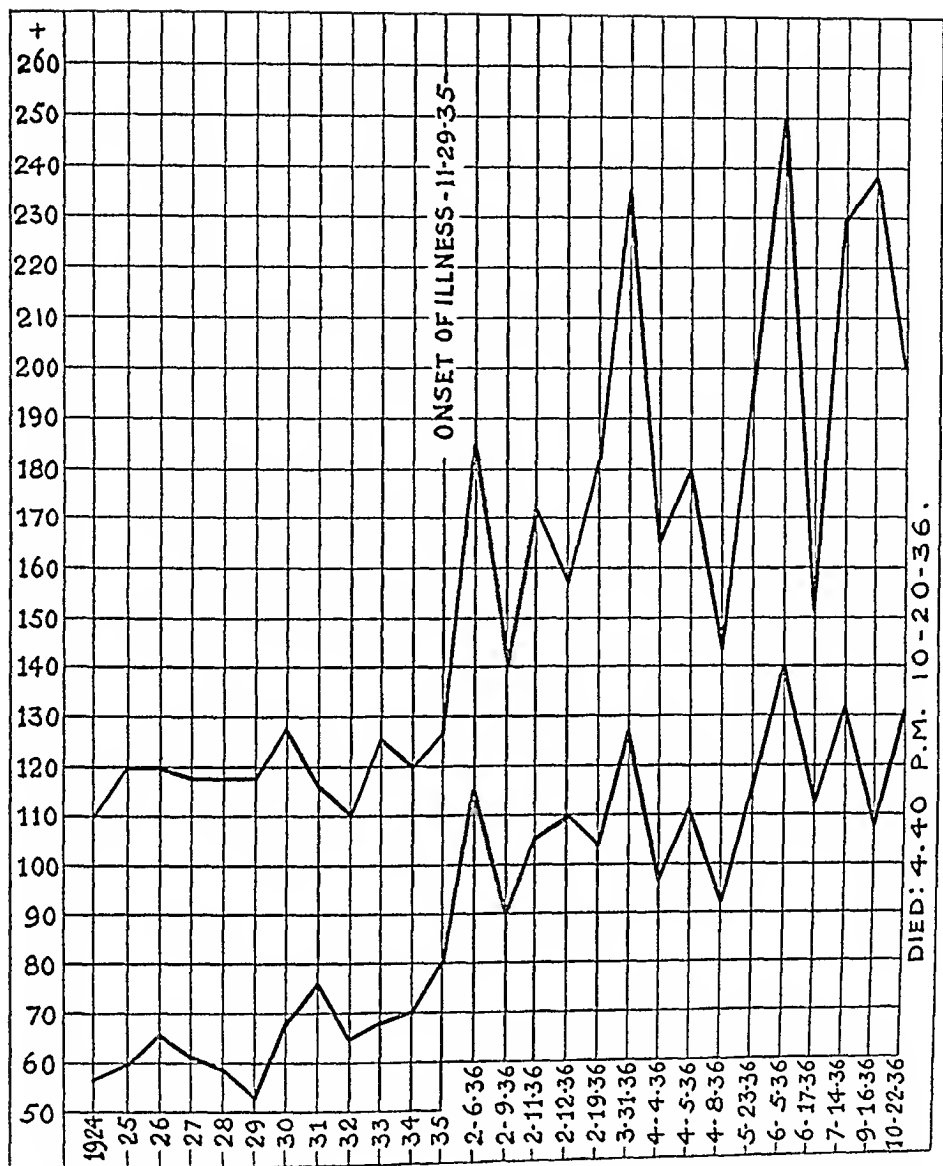
*Adrenals:* The adrenals were of normal size.

\* A-1612, Walter Reed General Hospital, Washington, D. C.



GRAPH 1. Blood pressures, Case 1: essential hypertension of the benign type, death from cerebral accident (A-1612, W.R.G.H.).

The graph shows: (1) At no time was the pulse pressure within the normal limits indicated in Table I; from 1918, when the patient was 29 years old, to 1926 when he was 37 years old, the pulse pressures varied between 48 and 55 mm. Hg. (2) For a period of 3 years, 1926-1928 inclusive, the patient's diastolic pressure rose while the systolic pressure remained stationary and the pulse pressure dropped accordingly. (3) After 1929, when the patient was 40 years old, the diastolic and the systolic pressures rose concomitantly, fairly slowly at first, then with extreme rapidity up to the time of death which occurred Feb. 24, 1934.



GRAPH 2. Blood pressures, Case 2: paroxysmal hypertension, death from cerebral accident (A-2018, W.R.G.H.).

The graph indicates a normal blood pressure from the time of the patient's entry into the Service (1924) until the onset of the final illness, November, 1935, with the exception of a slight transitory rise during 1930 to 1931. After November, 1935, the blood pressure tracing resembles the temperature chart in a case of septicopyemia, marked by daily and hourly elevations and depressions. The final illness lasted approximately 11 months; death occurred Oct. 23, 1936 (the graph indicates the date of death as of Oct. 20, 1936, by error); cause of death, cerebral accident, hemorrhage into the optic radiation of the left hemisphere of the forebrain.

*Kidneys:* The right kidney weighed 110 gm., the left 120 gm.; combined renal weight 230 gm. The weight of the heart (550 gm.) divided by the combined renal weight (230 gm.) was 2.39 (ratio). The cortical surfaces of the kidneys were pinkish and granular; the renal cortices measured 5 mm. in thickness and the markings were preserved. The sinus fat was slightly increased. There were several mulberry-like calculi located in the calices of both kidneys and an impacted calculus was lodged in the lumen of the right ureter.

Microscopic examination of the renal tissue revealed moderately advanced nephrosclerosis of the benign type involving the interlobular and interlobar arteries. The afferent glomerular arterioles were not involved.

*Brain:* The brain weighed 1610 gm. The convolutions were flattened. The left lateral ventricle was distended with clotted blood due to a "blow-out" hemorrhage involving the posterior cornu of the left internal capsule with extensive spread through the adjacent parietal and frontal lobes of the forebrain and rupture into the left lateral ventricle. The hemorrhage was single. The vertebral arteries, the basilar artery, the circle of Willis and the cerebral branches all presented thickened parchment-like walls with encircling atheromatous bands.

*Anatomical Diagnoses:* (1) Eccentric hypertrophy of the heart, weight of heart 550 gm.; (2) atheromatosis of the aorta, moderately advanced; (3) coronary arteriosclerosis, moderately advanced without obstruction; (4) cerebral arteriosclerosis, advanced; (5) cerebral hemorrhage, massive, source of hemorrhage the left lenticulostriate artery, with destruction of the posterior cornu of the left internal capsule and rupture into the lumen of the left lateral ventricle of the brain; (6) renal arteriosclerosis, benign type, moderately advanced (microscopic finding); and (7) nephrolithiasis, bilateral, with impacted calculus in the lumen of the right ureter.

### *Comment*

The blood pressure readings of the above case, from May 18, 1918, to death, were secured and are incorporated in Graph 1.\*

\* Through the courtesy of Major E. S. Cooley, U. S. Army, Office of the Surgeon General, Washington, D. C.

Study of the graph brings out the following points. (1) At no time was the patient's pulse pressure within the normal limits indicated in Table I. From 1918 when the officer was 29 years old, to 1926 when he was 37 years old, his pulse pressure varied between 48 and 55 mm. Hg. (2) For a period of 3 years, 1926 to 1928 inclusive, the patient's diastolic pressure rose while the systolic pressure remained stationary and the pulse pressure dropped accordingly. (3) After 1929, when the officer was 40 years old, the diastolic and the systolic pressures rose concomitantly, fairly slowly at first,

TABLE I  
*Data on Body Weight and Blood Pressure of 256 Army Officers*

Decade	Number of cases	Body weight	Systolic blood pressure	Diastolic blood pressure	Pulse pressure
		<i>lbs.</i>	<i>mm. Hg.</i>	<i>mm. Hg.</i>	<i>mm. Hg.</i>
3rd	37	150	120	74	46
4th	51	160	122	77	45
5th	97	166	124	79	45
6th	64	161	132	81	51
7th	7	158	138	84	54
<i>Averages:</i>	256	159	127	79	48

NOTE: The ages vary from 24 to 64 years, the retirement age of army officers. The diastolic pressures present a fairly uniform rise with an increase of 10 mm. Hg. in 40 years, an average increase of 0.25 mm. Hg. per year, diastolic blood pressure.

then with extreme rapidity up to death, which occurred February 24, 1934.

The results of the annual physical examinations of 256 army officers for the year 1939 were made available for study\* and are found to include valuable data relative to the blood pressure readings of a group of white males who are under constant medical supervision and whose lives and pursuits are fairly uniform. Of the total 256 officers 14, or 6 per cent, classify as obese, body weights averaging 90 Kg.; 6, or 2 per cent, present evidence of essential hypertension, not regarded as of sufficient severity to justify retirement; and 3, or 1 per cent, present some of the physical manifestations of the senile type of general arteriosclerosis. However, for the purpose intended, to supply workable averages, no cases were excluded and the body weights and the blood pressures of the 256 army officers are listed in Table I.

\* Through the courtesy of Lt.-Colonel D. H. Mebane, Medical Corps, U. S. Army, Chief of the Medical Service at the Tripler General Hospital, Honolulu, Hawaii.

## PART II

## PAROXYSMAL HYPERTENSION

*The Adrenal Glands*

In order to comprehend the pathology of the adrenal gland one must be reasonably cognizant of the histogenesis, anatomy and pathology of that organ.

*Histogenesis:* Quoting Maximow<sup>2</sup>: "The cortex develops from the celomic mesoderm on the medial side of the wolffian ridge, and the medulla from the ectodermal tissue from which the sympathetic ganglion cells also arise." Quoting Bailey and Cushing<sup>3</sup>: "The cells of the ganglionic crest . . . migrate into the visceral areas to form the anlage of the sympathetic nervous system. When they reach their final stations they differentiate into the neurons of the sympathetic ganglia, the capsular cells of these ganglia, and into the *chromaffine* cells of the adrenal medulla and analogous structures."

*Anatomy:* The adrenals are wedge shaped organs located on the upper poles of the kidneys. At birth the adrenals are relatively large, approximately one-third of the renal weight; during adult life the combined adrenal weight, ranging from 10 to 20 gm., is approximately one-thirtieth of the combined weight of the kidneys. The adrenals are located in a mesh of nerve fibers derived from the celiac ganglia, splanchnic, vagi and phrenic nerves. The adrenals receive their blood supply from three sources: from the inferior phrenic arteries, directly from the abdominal aorta, and from the renal arteries. The adrenal arteries break up into arterioles which course throughout the fibrous capsules of the adrenals; the adrenal arterioles reflect any degree of arteriolar sclerosis which may be found during the microscopic examination of the renal tissue. The arterioles in the adrenal capsules divide into capillaries which course inward between the lines of cortical cells to form the anastomosing capillary mesh of the *formatio reticularis*; the capillaries then reunite to form the sinusoids of the adrenal medulla. In the adrenal medulla the sinusoids join to form one large vein on either side, emptying into the inferior vena cava on the right and into the renal vein on the left, almost directly

below the eustachian valve of the heart. The walls of the two adrenal veins have a tunica media composed of longitudinally arranged fasciculi of smooth muscle fibers. In hypertension, chronic or due to any cause, the muscle fasciculi of the adrenal veins hypertrophy. Nervous stimuli to the adrenals pass: (1) to the chromaffin cells through the multipolar sympathetic neurons located in the substance of the adrenal medulla; and (2) to the longitudinal bundles of smooth muscle cells forming the walls of the adrenal veins, exciting a shortening of the veins with a pulling or suction action on the soft vascular tissue of the adrenal medulla, directly aiding the withdrawal of the secretory product (epinephrin) into the lumen of the sinusoids and veins, to be poured almost directly into the right atrium of the heart.

At birth the glomerular zone of the adrenal cortex is well developed; during adult life it gradually undergoes sclerosis, and at senility is hardly recognizable. Approaching senility the fascicular zone of the adrenal cortex undergoes nodular hyperplasia, at times quite marked though of no pathological significance. In essential hypertension the adrenal cortex is usually deep and the cells rich in lipoids. At birth the adrenal medullary parenchyma is sparsely represented though gradually increasing in relative volume as age advances. In cases of long continued hypertension from any cause the adrenal medullary parenchyma is increased in amount; the chromaffin cells show augmented physiological activity, manifested by the presence of numerous hyaloid cell inclusions located: (a) within the bodies of the large basophilic cuboidal and cylindrical cells palisading on the thin walled sinusoids of the adrenal medulla; and (b) within the capsules and in the bodies of the sympathetic neurons.

*Physiology:* The adrenal cortical parenchyma is indispensable for the continuance of life; adrenalectomized animals die after passing into a state of hemoconcentration with cationic imbalance; and there is marked increase in sodium elimination and potassium retention. Quoting Anderson and coworkers<sup>4</sup>: "Cushing's syndrome is an expression of the overproduction of cortin; studying the urine excretion of sodium and potassium and the blood level of these electrolytes, and comparing the findings with those of a patient with Addison's disease, it is reasoned that since some patients with Addison's disease are helped by a high sodium and



low potassium intake, then patients with Cushing's syndrome should be benefited by being placed on the opposite regimen, namely low sodium and high potassium intake." These authors state that this form of treatment proved effective in 1 case; the blood pressure decreased and there was a loss in weight.

*Physiology of the Medulla:* Epinephrin is the secretory product of the adrenal medullary parenchyma. The most delicate biological reaction, positive to a 1:800,000,000 concentration, fails to demonstrate any increase in the amount of epinephrin in the circulating blood. Quoting Rogoff and Marcus<sup>5</sup>: "Intravenously injected epinephrin disappears from the circulation so rapidly that it is rarely detected beyond the point of its pressor action and not after one complete circulation of the blood." The above observer believes that the concept that hypersecretion of epinephrin causes persistent elevation of blood pressure is untenable.

The normal physiological action of epinephrin may possibly be regarded as a direct action on the auriculoventricular node of the conductive system of the heart, located above the base of the septal cusp of the tricuspid valve. In paroxysmal hypertension resulting from adrenal medullary tumors of the pheochrome cell type the liberation of epinephrin during the episodes or crises exceeds all physiological and experimental bounds.

The correlation between the physiological functions of adrenal and thyroid is illustrated by 2 cases coming to autopsy. In 1 case, a white female,\* aged 74 years, the thyroid weighed 55 gm., being five or six times the usual weight in senility, and it was rich in colloid. The adrenals presented complete bilateral destruction of the cortical parenchyma and most of the medullary portions by tuberculous caseous-nodose lesions. (2) In a 2nd case, a white male,† aged 48 years, there was complete replacement of the thyroid parenchyma by neoplastic tissue, carcinoma solidum. The combined weight of the adrenals was 45 gm., over twice the normal maximum weight of these organs. Another case,§ which illustrates the physiological function of the adrenal cortical parenchyma, was that of a white male, aged 39 years: the thyroid weighed 32 gm. (normal); the kidneys were polycystic, combined weight

\* A-1802, Walter Reed General Hospital, Washington, D. C.

† A-1803, Walter Reed General Hospital, Washington, D. C.

§ A-2213, Walter Reed General Hospital, Washington, D. C.

7620 gm.; the combined adrenal weight was 26 gm., and the adrenals showed marked cortical hyperplasia regarded as the effort to maintain the cationic balance between the blood plasma and the blood and tissue cells of the patient.

*Pathology:* The pathology of the adrenal gland may be divided into: (a) lesions primary in the adrenal itself, cortical and medullary portions; and (b) lesions primary in the adrenal retroperitoneal rests, the organs of Zuckerkandl, and adrenal rests in the substance of the kidneys, the primary foci of Grawitz tumors. Adrenal cortical cell rests are sometimes encountered attached to the epididymes of the testes, but I have no record of primary tumors of adrenal tissue in this location. Limiting the discussion of adrenal neoplasia to that primary in the medullary parenchyma, the 1105 autopsies forming the basis of this study furnish two examples: (1) a neuroblastoma\* having as its chief cell a unipolar neuroblast, a cell midway in its developmental position between the undifferentiated cells migrating from the ganglionic crest of the embryo and the highly differentiated chromaffin cells and sympathetic neurons of the adrenal medulla; and (2) a 2nd case,† a pheochromoblastoma, in which the chief cell was a variant of the highly differentiated chromaffin cells of the fully developed adrenal medullary parenchyma. As would be expected from their respective developmental positions, the neuroblastoma was highly malignant, widely metastasizing and without physiological activity, while the pheochromoblastoma was relatively benign, showing only local invasion and extension and presenting a very highly developed physiological activity manifested by episodes of hypertension of the paroxysmal type.

#### *Abstracts of Reported Cases of Paroxysmal Hypertension*

The medical literature includes reports of a number of cases of paroxysmal hypertension and the following case reports will be briefly abstracted.

Belt and Powell<sup>6</sup> described a syndrome characterized by paroxysmal hypertension and periodic tachycardia, pallor followed by a flushing of the skin, glycosuria, headache, nausea and vomiting, and pulmonary edema. The case reported is that of a Jewess aged

\* A-1718, Walter Reed General Hospital, Washington, D. C.

† A-2018, Walter Reed General Hospital, Washington, D. C.

45 years. The patient died during a crisis accompanied by marked pulmonary edema. Autopsy revealed an adrenal tumor weighing 1000 gm. The chemical examination demonstrated 0.2 gm. of epinephrin per 100 gm. of tissue. The technic of the colorimetric estimation of epinephrin was that of Folin, Cannon and Denis.<sup>7</sup>

Labbé, Tinel and Doumer<sup>8</sup> report the case of a female, aged 28 years, who suffered from attacks of nausea and vomiting, three to four attacks a week. During these attacks the systolic blood pressure rose to 280 mm. Hg. Glycosuria was noted. The hypertension varied from day to day and from hour to hour. Pulmonary edema developed and the patient died. The left adrenal was replaced by a tumor mass about 6 cm. in diameter.

Labbé, Violle and Azérad<sup>9</sup> report a case of "paroxysmal sympathicotonia" in a male patient aged 29 years, who died as the result of cerebral hemorrhage.

Mayo<sup>10</sup> reports the case of a female aged 30 years. The chief symptoms were nausea and vomiting, and cough with blood tinged expectoration. The crises were marked by paroxysmal hypertension during which the systolic blood pressure varied between 300 and 180 mm. Hg. The patient was operated upon and a pheochromic tumor (paraganglioma) was removed. The tumor mass measured 6 by 4 cm. and was located retroperitoneally mesial to the left kidney. The patient made a complete recovery.

Rabin<sup>11</sup> reports the case of a Polish woman aged 45 years. The clinical course was marked by episodes characterized by nausea and vomiting with rise in blood pressure up to 226/180 mm. Hg. Coma developed and the patient died. At autopsy an adrenal medullary pheochromocytoma was found which yielded 1.5 mg. of epinephrin per gm. of tissue.

Burgess, Waterman and Cutts<sup>12</sup> report the case of a Jewess, aged 25 years, who complained of palpitation associated with pallor and constipation. At the onset of the crises the pulse rose to 144 per minute; bradycardia accompanied by extrasystoles developed. Headache was severe. The pyelogram demonstrated a downward displacement of the right kidney. The blood sugar was 125 mg. per cent, and the non-protein nitrogen 31 mg. per cent. The Wassermann test was negative. The patient was operated upon and died almost immediately from pulmonary edema. During operation a tumor mass 9 cm. in diameter, soft and hemorrhagic,

was removed from the upper pole of the right kidney. The microscopic diagnosis was pheochromocytoma primary in the medulla of the right adrenal. No metastases were found.

Geschickter <sup>13</sup> reports a "benign paraganglioma" of suprarenal medullary tissue in a woman aged 53 years. Geschickter states: "In paragangliomas of the suprarenal glands, hypertension, hypotension, and vasomotor instability are not infrequently observed . . . the suprarenal cortex may be invaded and destroyed. . . ."

Ewing <sup>14</sup> classifies adrenal tumors as cortical and medullary. Medullary tumors of the adrenal are subgrouped as focal hyperplasia, neuroma ganglionare, neuroblastoma, and chromaffin cell tumors. He states that medullary chromaffin cell tumors form large masses. The sinuses are invaded but there are no metastases. The structure is composed of groups of round, oval and polygonal cells, most of which are small. Giant cells with single or multiple nuclei occur. Pigment is irregular in distribution. Homogeneous acidophilic cell inclusions are usually present.

Shipley <sup>15</sup> reports the case of a young married woman, aged 26 years, who complained of attacks characterized by cardiac palpitation, nausea and vomiting. Severe occipital headache was an increasingly troublesome symptom. Her blood pressure varied from 120/90 on admission, to 190/98, rising quickly to 219/110 and falling rapidly to 176/76 mm. Hg. The diagnosis of adrenal tumor was made but the side was not determined. The patient was operated upon, first on the left side, and the left adrenal was found to be normal; on the right side a tumor mass primary in the right adrenal was discovered and removed. The day following the second operation the patient improved. During convalescence the blood pressure remained low, 116/74 mm. Hg. Ten months later she reported herself to be free from previous symptoms. The mass removed weighed 115 gm. A small amount of cortical adrenal tissue remained extending over one side of the neoplasm. Microscopic sections showed the tumor to be composed of oval and polygonal cells arranged in small alveoli separated by capillaries. The diagnosis was paraganglioma.

In my opinion the term paraganglioma should be limited to tumors arising from retroperitoneal rests of adrenal medullary tissue, while neoplasia primary in the adrenal medullary parenchyma should be designated as pheochromocytoma or pheochromo-

blastoma, depending on the degree of differentiation manifested by the tumor cells.

Kelly and his associates <sup>16</sup> suggest the following reasons for the paroxysmal nature of the hypertension associated with pheochrome cell tumors of adrenal medullary tissue: (a) periodic hemorrhages into the substance of the tumor mass; (b) any unusual exertion, such as bending, expressing epinephrin from the neoplastic tissue; and (c) emotional reactions such as are believed to stimulate an increased output of epinephrin. They are of the opinion that the complete absence of signs of arteriosclerosis is of particular interest; this is evidence against the conception that epinephrin plays any part in the production of arteriosclerosis and essential hypertension. I do not entirely concur with the foregoing assumption; although epinephrin probably does not play a primary rôle in essential hypertension, still I believe that a secondary physiological function is maintained by the adrenal medullary parenchyma in essential hypertension. They report the case of a married woman, aged 37 years, who complained of episodes during which the blood pressure rose from 90/70 to 280/160 mm. Hg. During these episodes extreme cutaneous pallor developed and the patient was nauseated. The attacks lasted about 15 minutes. The pulse was not appreciably accelerated. The blood sugar was 118 mg. per cent. Indefinite resistance and tenderness were elicited above the right kidney, which was palpable. The patient was operated upon and a tumor mass was located cephalad to the upper pole of the kidney. The mass was 10.5 cm. in diameter and weighed 240 gm. On chemical examination the neoplastic tissue yielded approximately 300 mg. of crystalline epinephrin. Postoperatively the convalescence was uneventful and no further episodes occurred.

Evans <sup>17</sup> reports the case of a girl, aged 12 years, who died during an episode of paroxysmal hypertension. Autopsy revealed a pheochrome cell tumor primary in the medullary portion of the left adrenal.

Wells and Boman <sup>18</sup> note that the characteristic symptoms of 13 patients completely disappeared following surgical removal of benign tumors composed of epinephrin-producing cells such as are normally present in the adrenal medulla. They state that during severe attacks there may be failure of the left ventricle of the heart, manifested by cyanosis, coughing with blood tinged sputum,

and that the sudden transient spasm of the arterioles of the body accounts for many of the manifestations of this condition. The attacks tend to increase in severity and frequency. They report the case of a female, a school teacher aged 30 years. The initial symptom was a pounding of the heart like a sledge hammer. During these attacks the blood pressure rose to 180/140 mm. Hg. and then dropped suddenly. The patient died during anesthesia for attempted appendectomy. Autopsy demonstrated a tumor primary in the right adrenal and weighing 20 gm. Chemical examination of the neoplastic tissue showed 1 per cent of epinephrin.

The 12 cases of pheochromocytoma, including the following case, may be classified as follows: females, 10 (2 Jewesses); males, 2; average age 34 years. Two of the tumors were primary in retroperitoneal rests of adrenal medullary tissue (paragangliomas); 5 were primary in the right adrenal medulla and 2 in the left; in 3 of the cases abstracted the side involved is not indicated. In 3 cases transient glycosuria was noted. The 2 male patients died as the result of cerebral hemorrhage. Three of the patients died during crises as the result of marked pulmonary congestion and edema. Four of the female patients were operated upon; 1 died immediately following operation, and 3 made complete post-operative recovery without subsequent episodes.

### *Case 2. Paroxysmal Hypertension*

*Clinical History:* The patient, A. D. M., was a white male, a veterinarian of the Medical Department of the U. S. Army. There were no previous illnesses other than the usual diseases of childhood. He used no alcohol and smoked in moderation. The family history revealed no chronic cardiovascular disease. The patient was admitted to the hospital Nov. 15, 1929, stating that he had passed a large tarry bowel movement. On admission to the hospital he was pale and anemic in appearance. The blood pressure was 108/60 mm. Hg. He was treated on the basis of an emergency duodenal ulcer and was discharged on March 17, 1930.

The patient had enjoyed good health from March, 1930, to November, 1935. On Nov. 29, 1935 he caught cold, complained of headache and was nauseated. He was hospitalized, placed on the Sippy regimen, and discharged later improved. On December 7th he suddenly felt cold and his head ached. Within a few hours he became delirious for about 48 hours, and after complete consciousness returned he remarked that his vision had become hazy. The blood counts, the Wassermann reaction, the spinal fluid examinations and the results of blood chemistry were all negative. The blood pressure on Jan. 5, 1936 was 172/118 mm. Hg.

The patient was transferred to the Walter Reed General Hospital, Washington, D. C., on Feb. 4, 1936. At this time he was 45 years old. On admission

the pupils were equal and reacted to light and accommodation. The blood pressure was 154/96 mm. Hg. On February 7th, after an enema, the patient became nauseated and complained of severe headache; following catharsis (castor oil) the headache subsided and he felt better. On March 4th nausea and vomiting recurred accompanied by severe headache. A spinal tap was made; the spinal fluid was clear, pressure 20 mm. Hg. A brain tumor was considered as a possibility. On March 16th the urine became bloody; the clinical record states that "hypernephroma must be considered." On March 19th the urine was free from blood. On March 23rd the case was considered as one of "undoubtedly essential hypertension with renal arteriolar sclerosis." On March 27th hematuria occurred again and epididymitis (left) developed. By April 18th the patient's condition improved; he had gained in weight and was out of bed in a wheel-chair. On May 8th the headaches returned and became increasingly severe; he was nauseated and vomited frequently. The clinical record on June 5th notes: "The patient remarked that he felt better after saline infusions." On July 13th another crisis developed; the clinical notes state: "The distressing spells are coming on more frequently; the attacks arise when the patient is constipated and are initiated by headache, nausea and vomiting. The subjective symptoms are relieved by saline infusions and by castor oil. During the attacks the extremities are cold." On October 22nd an attack began; the patient vomited and became restless and irrational. There was involuntary urination. The blood pressure registered 200/130 mm. Hg. The urea nitrogen was 15 mg. per cent. The patient closed his right eye with difficulty. The next day right hemiplegia developed; the pulse became feeble and the respirations stertorous. The patient died at 4.40 P.M. on Oct. 23, 1936. The duration of the final illness was approximately 11 months.

The variations of the blood pressure readings are depicted in Graph 2. Throughout the final illness there was no elevation of temperature except during the last 2 days when the temperature rose to 102° F. The pulse varied between 80 and 110 beats per minute. At no time during the final illness were abdominal pain and tenderness elicited.

*Laboratory Data:* A roentgenogram on Feb. 6, 1936, showed that the cardiac shadow was normal in size, shape and position; the lungs were essentially normal. On August 31st flat plates of the abdomen were negative. No pyelograms were made. Some of the specimens of urine revealed 2 plus albumin, sugar negative, many leukocytes and erythrocytes, but no casts. The blood count on February 5th revealed: erythrocytes 3,960,000; hemoglobin 70 per cent; total leukocyte count 10,000; polymorphonuclears 68 per cent; eosinophils 3 per cent.

### *Postmortem Examination \**

The body was that of a poorly nourished white male, aged 45 years. The body length was 176 cm., the weight approximately 55 Kg. The voluntary musculature was fairly well developed. The pupils were equal at 3 mm. The neck and chest were of normal contour. The anterior abdominal wall was flat. No edema of the feet or ankles was present.

\* A-2018, Walter Reed General Hospital, Washington, D. C.

*Thyroid:* The thyroid weighed 29 gm. On microscopic examination the acini were large and the colloid homogeneous. The epithelium was of the cuboidal and flat types. There was no lymphocytic reaction. No thymus rest was encountered.

*Lungs:* Microscopic examination of the pulmonary tissue revealed an acute bronchiolitis and peribronchiolitis.

*Heart:* The heart weighed 385 gm.; the subepicardial fat was scant in amount. The wall of the left ventricle measured 2.2 to 1.5 cm. in thickness. The aortic ring was 7.3 cm. in circumference; the mitral ring 10 cm. The valves were normal. The coronary arteries and the aorta presented moderate intimal thickening, but no lime salt deposits or ulceration were present. The thoracic aorta measured 5.3 cm. in circumference and the abdominal aorta 4 cm.

*Stomach and Intestines:* Normal in appearance. The colon contained large masses of hard fecal material.

*Spleen:* The spleen was small, weighing 50 gm.

*Adrenals:* The left adrenal weighed 7 gm. and was normal in size and contour. The retroperitoneal fat surrounding the left adrenal included small aberrant groups of adrenal cortical parenchyma. On microscopic examination the cells of the left adrenal cortex were rich in lipoids and the medullary portion showed no alteration from the normal.

*Tumor Mass Replacing the Right Adrenal:* A tumor mass, 12 by 9 cm. in diameter, weighing approximately 200 gm. (section of the inferior vena cava is included in the gross specimen illustrated in Figure 1) was present in the right adrenal. The mass shelled out easily from the surrounding fat but was firmly united to the wall of the inferior vena cava and to the thickened overlying parietal peritoneum. Neoplastic tissue appeared in the lumen of the cava in the form of a nodule about 0.5 by 2 cm. The outer surface of the tumor was coarsely nodular, the consistence firm, and the cut surfaces brownish gray. After remaining in Kaiserling I the neoplastic tissue assumed a darker brown hue. The free surface of the parietal peritoneum overlying the tumor mass presented innumerable nodules varying in size up to 0.25 cm. The cut surfaces of the peritoneal nodules had the same gross appearance as the cut surfaces of the primary growth. Similar nodules were found on the under surface of the right leaf of the diaphragm



cephalad to the right adrenal tumor mass, and attached to the free surfaces of both leaves of the mesentery of the small intestine.

*Microscopic Examination of the Newgrowth:* The growth is very cellular; the cells are arranged in groups (alveoli), round to oval in contour, and are located in close proximity to thin walled blood channels (Figs. 2-4). The cells are quite pleomorphic, fusiform, cylindrical and pear shaped, and in some instances are united to the supporting fibrillary scaffolding by stem-like protoplasmic processes. The cells vary greatly in size and shape. The cytoplasm of the cells is amphophilic and finely granular; some of the cells contain unstained vacuoles and in other cells there are acidophilic hyaloid inclusions. The nuclei of the tumor cells are mostly small, circular in outline and eccentric in position; the linin network is poorly differentiated. A few cells present larger oval, hyperchromatic nuclei and scattered cells have two or more nuclei. Many of the neoplastic cells present an atypical nuclear division; the nuclear chromatin is divided into from 6 to 50 hyperchromatic bodies, oval, rod and dumbbell shaped and massed in the central zones of the dividing nuclei. In scattered areas throughout the newgrowth the neoplastic tissue contains a dull brown, coarsely granular pigment; the pigment gives a negative iron reaction with potassium ferrocyanide and is consequently regarded as melanin. In sections stained by Bielschowsky's method the fibrillar stroma includes no argentaffin fibrils; the tumor cells are silver-positive, and the cytoplasm of the cells appears as a fine silver gray dust including coarser particles of reduced silver.

*Kidneys:* The right kidney weighed 145 gm., the left 140 gm.; combined renal weight 285 gm. The weight of the heart (385 gm.) divided by the combined renal weight (285 gm.) equals 1.35, as compared with the cardiorenal ratio of 2.39 in the case of essential hypertension reported above.

*Urinary Bladder:* The wall was thickened and the trabeculae prominent. No cellulose formation was present. The bladder mucosa was studded with innumerable pale gray, slightly raised points the size of the head of a common pin. The trigone was low and narrow and the ureteral orifices normal. The microscopic picture presented by the bladder mucosa indicated a chronic follicular cystitis.

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